

Leaf Surface Sesquiterpene Alcohols of the Potato (*Solanum tuberosum*) and Their Influence on Colorado Beetle (*Leptinotarsa decemlineata* Say) Feeding

BEATA SZAFRANEK,^{*,†} KAROLINA CHRAPKOWSKA,[†] DANUTA WALIGÓRA,[‡]
 RICHARD PALAVINSKAS,[§] ARKADIUSZ BANACH,[†] AND JANUSZ SZAFRANEK[†]

Faculty of Chemistry, University of Gdańsk, 80-952 Gdańsk, Sobieskiego 18, Poland, Plant Protection Institute, 60-318 Poznań, Miczurina 20, Poland, and Bundesinstitut für Risikobewertung, Berlin, Germany

The structures of two previously unknown sesquiterpene alcohols of the potato (*Solanum tuberosum*) were assigned. The potato alcohols were obtained by steam-distillation, preparative column chromatography, and separation into fractions by HPLC on a silica gel column. The fractions were studied by GC-FID, GC-MS, and NMR spectroscopy. The potato sesquiterpene alcohols were identified as kunzeaol (6- α -hydroxygermacra-1(10),4-diene) and ledol. These two compounds were used in feeding tests with larvae and beetles of the Colorado potato beetle (*Leptinotarsa decemlineata* Say). In a bioassay, kunzeaol was found to act as a feeding attractant for the beetles.

KEYWORDS: *Solanum tuberosum*; potato; sesquiterpene alcohols; HPLC; NMR; Colorado potato beetle; feeding behavior

INTRODUCTION

The potato (*Solanum tuberosum*) is a very important food crop in many parts of the world. The plant is known to produce and emit a large variety of volatile organic compounds, among which monoterpenes, sesquiterpenes, and oxygen-containing terpenoids are dominant. Potato terpenes are mostly products of the glandular trichomes in the leaves. In recent years, their structures have been thoroughly investigated as they are implicated in the defensive and attractive roles of potato plants.

The relationship of Colorado potato beetles (CPB) to the host plant has been comprehensively investigated, and potato plant volatiles have been found important as regards the behavior of the insects. Most of the volatiles emitted by potato plants have been identified (1–3), and the insects' olfactory response to them has been investigated as well. Dickens (4) recently published a study on the molecular basis of potato–CPB interactions.

The separation and identification of terpenes rely heavily on gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS) analyses, but in some cases, these techniques are not sufficient for unambiguous identifications. This is because many sesquiterpenes have essentially similar skeleton structures, due to which their electron impact (EI) fragmentations yield similar mass spectra. This is also true of those sesquiterpene alcohols in the potato leaf surface whose

structures have not yet been assigned. NMR spectroscopy is therefore the method of choice for analyzing the compounds, since it is capable of establishing their relative stereochemistry.

In an earlier study of the foliar sesquiterpenes of *S. tuberosum* (5), we reported on two unknown sesquiterpene alcohols (I and III). The tentatively identified alcohols I and III were present in significant amounts (0.6–35 ng/cm² and 0.2–94 ng/cm², respectively) in the leaf surfaces of 10 potato varieties. The sesquiterpene compositions of 8 potato varieties were dominated by high contents of alcohol III. The aim of the present study was to identify these alcohols and to determine their bioactivity as feeding attractants/deterrents to the Colorado potato beetle.

MATERIALS AND METHODS

Potato Plants. *Solanum tuberosum* plants (cv. Mila) were field-grown from certified seed tubers on plots near Gdańsk (Juszkowo, 54°16'20" N 18°36'42" E). The seven-week-old plants were harvested, transferred immediately to the laboratory, weighed, and steam-distilled or extracted.

Steam-Distillation and Extraction. Potato leaves (3 kg) were steam-distilled for 1 h. The distillate was extracted three times with methylene chloride, and the organic fraction was dried with anhydrous sodium sulfate. The solution was concentrated at atmospheric pressure with a 10-cm distillation column and evaporated under a gentle stream of nitrogen. As an alternative, a sample of sesquiterpenes was prepared by extracting (10 s) potato leaves (~100 g) with methylene chloride.

Column Chromatography. The essential oil of potato leaves (3 × 47 mg) was separated on a 24 × 1 cm silica gel column. The MN-Kieselgel 60, 70–270-mesh silica gel was previously rinsed with methanol, acetone, and hexane, then dried at 160 °C for 8 h, and deactivated by the addition of 15% distilled water. The separation was

* Corresponding author: telephone, +48 58 3450351; fax, +48 58 3450472; e-mail, beata.szafranek@univ.gda.pl.

[†] University of Gdańsk.

[‡] Plant Protection Institute.

[§] Bundesinstitut für Risikobewertung.

carried out at a temperature of 10 °C. The fractions were sequentially eluted with 50 mL of pentane, 20 mL of methylene chloride, and finally 25 mL of methylene chloride to provide the mixture of sesquiterpene alcohols. The fractions were concentrated using a 10-cm distillation column, evaporated under a gentle stream of nitrogen, and analyzed by GC and GC-MS.

High-Performance Liquid Chromatography. HPLC-LSD separations of the potato sesquiterpene alcohols were performed on a 250 × 4.6 mm i.d. silica gel column (Alltech) attached to a Shimadzu chromatograph equipped with a light-scattering detector (LSD). The detector evaporation temperature was 40 °C, and the carbon dioxide pressure was 0.1–0.2 MPa. Isocratic elution with methylene chloride was applied at a flow rate of 0.6 mL/min. The separation was repeated 20 times. The fractions were evaporated and analyzed by GC-FID and GC-MS. The total quantities of the HPLC-separated alcohols were as follows: kunzeaol (5.2 mg, purity by GC-FID: 95%), and ledol (1.6 mg, 99%).

Isolation of the Ledol Standard from *Ledum palustre*. *L. palustre* plants were harvested in the Białowieża National Park (52°42' N 23°52' E) (Herbapol, Białystok, Poland). The dry aerial parts of *L. palustre* (400 g) were steam-distilled for 5 h. The distillate was neutralized with 1 g of NaHCO₃ and extracted three times with CH₂Cl₂. The essential oil was subjected to column chromatography separation on silica gel as described for the potato essential oil. The alcohol fraction derived from *L. palustre* was concentrated and separated by HPLC with a refractometric detector on a 250 × 4.6 mm i.d. Econosil C₁₈ reversed phase column (Alltech). Isocratic elution with acetonitrile was applied at a flow rate of 0.6 mL/min. The total quantity of HPLC-separated ledol was 3.7 mg; purity by GC-FID: 98%; *k'* = 1.84.

Nuclear Magnetic Resonance Spectra. NMR spectra were recorded for the sesquiterpene alcohols in deuteriochloroform (0.7 mL) on a Varian Mercury 400-MHz (kunzeaol) or a Varian Unity plus 500-MHz (ledol samples) FT-NMR spectrometer. All spectra were recorded at 295 K. Chemical shifts were measured relative to the residual solvent resonances at 7.27 ppm for the ¹H and at 77.0 ppm for the ¹³C spectra, respectively. Proton spectra were recorded with 6-kHz (400 MHz) and 8-kHz (500 MHz) spectral widths. Proton-decoupled ¹³C spectra, 100 MHz, were recorded with a 25000-Hz width for 9000 repetitions. Proton homonuclear-correlated two-dimensional spectra were performed for 256 (COSY, 400 MHz) and 512 increments (DGF-COSY, 500 MHz) with an accumulation of 16 transients for each step, over respective sweep widths of 2252.6 and 1658.65 Hz. Coupling constants *J*_{H,H} were obtained from DQF-COSY, later improved by multiplet analysis of ¹H signals. The approximate error did not exceed 0.5 Hz. The NOESY spectra (500 MHz) were measured with a 1658.65-Hz sweep width in both dimensions for 32 repetitions and 256 increments. The mixing time was 0.7 s. For the 2D ¹H–¹³C long-range heteronuclear shift correlations (HMBC), 400 increments were performed with the accumulation of 64 repetitions for 2294.1-Hz (¹H) and 24169.2-Hz (¹³C) sweep widths (400 MHz) and 512 repetitions in 140 increments over 4414.5-Hz (¹H) and 12569.6-Hz (¹³C) sweep widths (500 MHz). For the 2D ¹H–¹³C heteronuclear shift correlations (HSQC), 32 scans in 2 × 128 increments over 2252.8- and 16112.8-Hz sweeps and 128 scans in 2 × 130 increments over 1658.65- and 11312.6-Hz sweeps were collected for 400 and 500 MHz, respectively.

Gas Chromatography. The analyses were carried out on a GC 8000 TOP (CE Instruments) gas chromatograph equipped with a capillary column with a split ratio of 1:30 for the injection port and direct connection to a FID. The carrier gas was argon. For reliable sesquiterpene analysis, the injector and detector temperatures were 220 °C. The 30 m × 0.25 mm i.d. columns used (film thickness = 0.25 μm) were RTX-5 (Restek) and SolGel 1 (SGE). Retention indices (RI) were obtained from a temperature program (60–220 °C, rate 3 °C/min) on the RTX-5 column to match the conditions used by Adams (6). The co-injections with the ledol standard were performed on the RTX-5 and SolGel 1 columns under the conditions described above.

Mass Spectrometry. Mass spectra (70 eV) were recorded on an SSQ710 quadrupole mass spectrometer (Finnigan MAT). The samples were introduced through a Hewlett-Packard 5890 gas chromatograph equipped with the same columns and under the same chromatographic

conditions as for the GC analysis but with helium as carrier gas. The ion source was maintained at 180 °C.

High-resolution mass spectrometric data of the alcohol molecular ions *m/z* 222 (GC-MS) for elucidating the ion composition were recorded on a Finnigan MAT 95 double-focusing mass spectrometer (reverse geometry) at resolutions of 10000 to 20000 with the Selected Ion Recording technique (7). The PFK masses 218.98563 and 230.98563 were our “lock” and “calibration” masses bracketing the analyte masses.

Potato Leaf Disc Bioassay. The influence of potato sesquiterpene alcohols on the feeding of CPB adults and larvae was determined using potato leaf discs deprived of their surface components, in a choice bioassay during July and August. Kunzeaol (purity by GC-FID: 95%) and ledol (99%) were used in feeding tests. All experiments were conducted at room temperature (20–25 °C) and in natural daylight between 11 a.m. and 2 p.m. The assays were performed in series over 15 days with each sesquiterpene alcohol represented in each series to compensate for any variance in CPB quality and conditions between replications.

To provide insect material for the biological experiments, fourth-instar CPB larvae were collected in the growing season from potato fields near the research center (Poznań, Poland). The larvae were placed in sand-filled containers, where they continued feeding on fresh potato leaves (cv. Drop) at temperatures of ca. 20–25 °C and in a natural summer light–dark photoperiod. When the larvae stopped feeding and burrowed into the sand for pupation, the potato leaves were removed and the containers covered with bolting cloth. For the duration of pupation, the containers were maintained under the same conditions as described above. The newly emerged beetles were placed in cages with fresh potato leaves dipped in glass vials containing water. After at least 2–3 days of feeding, the beetles were transferred to the experiments.

The larvae for all experiments were obtained from the continual rearing conducted under the same conditions. Hatched from eggs laid by beetles collected from the field, first-stage larvae were reared on fresh potato leaves until they reached the third-instar stage, when they were transferred to the experiments.

Before the assay, both beetles and third-instar larvae had been starved for about 4 h. The insects were used only once in the experiments. The choice tests were carried out in 7-cm-diameter glass Petri dishes coated with moistened filter paper. The leaf discs (2.1 cm²) were cut from potato leaves (cv. Drop) with a cork borer and washed in methylene chloride (10 s), after which the solvent was completely evaporated from the leaf discs. The sesquiterpene alcohols were dissolved in methylene chloride. The upper surfaces of the potato leaf discs were treated with 40 μL of solution of the tested alcohol (test disc) or the solvent carrier alone (control disc). The amounts of sesquiterpene alcohols were about 5 times greater than those occurring naturally on potato leaf surfaces (kunzeaol, 1.2 μg on 2.1 cm² disc, and ledol, 0.3 μg on 2.1 cm² disc). After complete evaporation of the solvent, one control disc and one test disc were placed in one Petri dish, together with two beetles or two larvae. After 2 h (beetles) or 2.5 h (larvae) of feeding, the insects were removed from the dishes and the areas of the test and control discs measured using a computer program specially designed by the Plant Protection Institute. The bioassays were performed in 15 replications for each sesquiterpene alcohol.

The initial leaf disc area (before feeding) was determined from the mean area of a separate group of 10 solvent-extracted discs cut from the same leaves used in each assay, referred to as “initial discs”. Consumption was calculated by subtracting the area of the test disc or control disc from the area of the initial discs.

The consumed areas on the test and control discs were compared statistically using the Wilcoxon test for paired samples.

A preference index (PI) was used to express the data:

$$PI = (T - K)/(T + K)$$

where *T* is the consumed area of the test disc (cm²) and *K* is the consumed area of the control disc (cm²). Zero indicates no preferences, and +1 or –1 indicates strong attractant or deterrent activity, respectively.

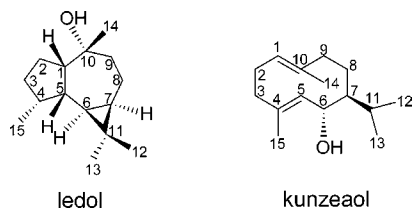


Figure 1. Structures of sesquiterpene alcohols, ledol and kunzeaol.

RESULTS AND DISCUSSION

Ledol Standard from *Ledum palustre*. Ledol (Figure 1) as a standard compound was obtained from *L. palustre* (Labrador tea, also known as *Rhododendron tomentosum*) by steam-distillation, then LC and RP-HPLC separations, and identified by GC-MS and NMR (Table 1). A literature survey of ledol has shown many confusing assignments, so its identification is

not a trivial matter (8). Ledol was first isolated from *L. palustre*, a plant common to sphagnum meadows (9). Only a compound with a structure identical to that obtained from *L. palustre* can be called ledol. We therefore decided to separate this compound from *L. palustre* in order to compare the chromatographic and spectroscopic data with that of potato sesquiterpene alcohol I. The molecular structure of ledol from the essential oil of *L. palustre* was elucidated by X-ray analysis (10), so its stereochemistry is known.

The NMR data of the easily crystallized sesquiterpene alcohol derived from *L. palustre* (Table 1) are virtually identical to those of Kaplan et al. (8) and Bombarda et al. (11). For signals assignments, two-dimensional experiments such as DQF-COSY, HSQC, and HMBC were used.

Structural Studies of Potato Sesquiterpene Alcohols. The identification of two leaf surface sesquiterpene alcohols in *S.*

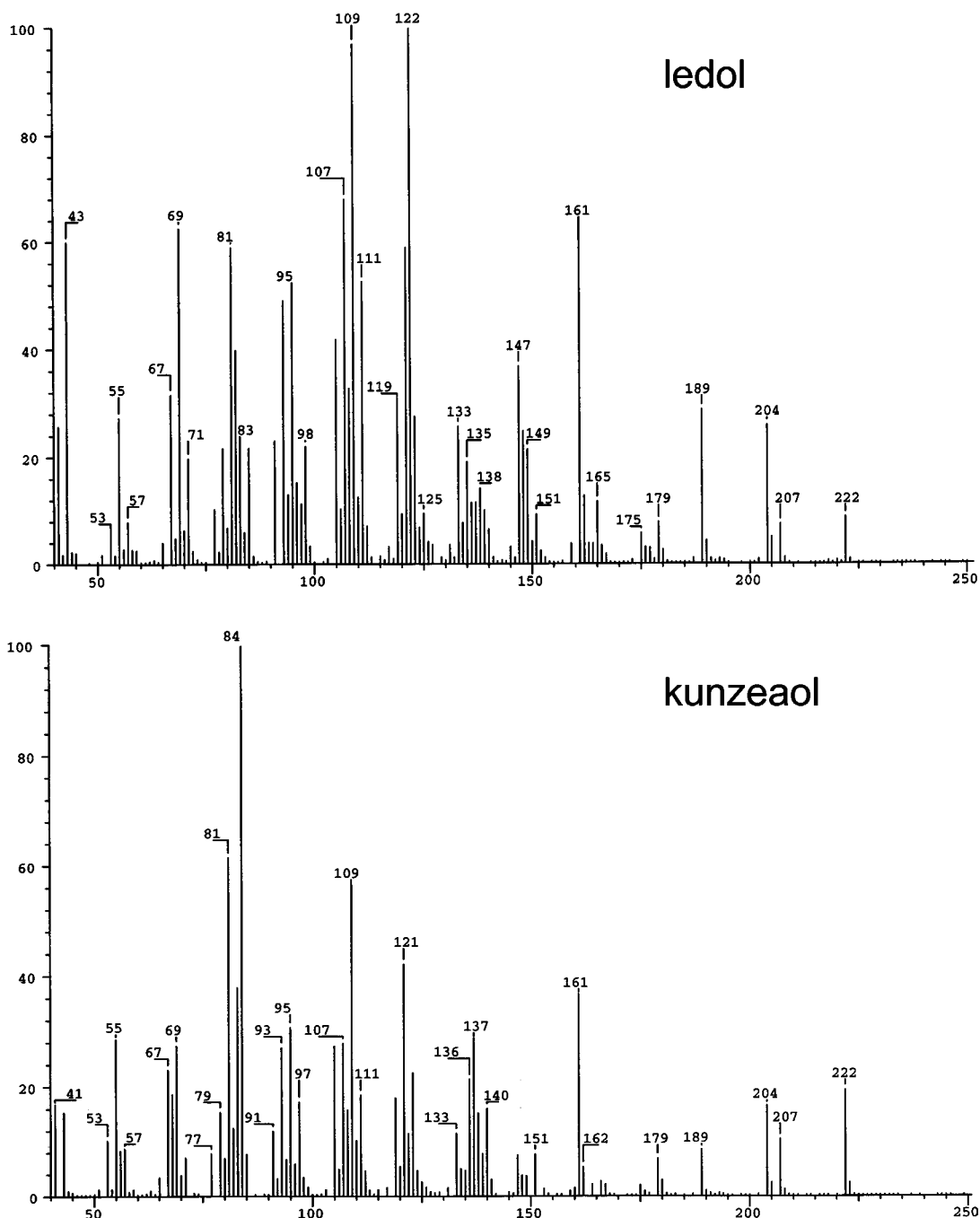


Figure 2. Mass spectra (70 eV) of sesquiterpene alcohols.

Table 1. NMR Spectroscopic Data^a for Ledol and Kunzealol

ledol					kunzealol ^b				
<i>S. tuberosum</i>					<i>L. palustre</i>		<i>S. tuberosum</i>		
No.	δ ¹³ C	δ ¹ H	J _{H,H}	NOE with H	δ ¹³ C	δ ¹ H	No.	δ ¹³ C	δ ¹ H
1	53.9	2.11	6.3	2 β , 5, 14	53.8	2.11	1	121.4 (128.8)	4.93 (4.94)
2 α	24.7	1.92		2 β , 3 α , 14	24.8	1.91	2	41.4 (35.8)	2.16 (2.34)
2 β		1.71		1, 2 α , 14		1.70	2		1.64
3 α	31.0	1.31		3 β , 15	31.0	1.30	3	37.1 (39.1)	2.09 (2.10)
3 β		1.72		3 α , 4		1.71	3		2.13
4	38.6	2.01	6.3	3 β , 5, 15	38.6	2.00	4	132.4	
5	40.8	1.80	6.3; 10.7	1, 4, 6, 8 β , 12, 14	41.0	1.79	5	131.5 (133.6)	4.98 (5.07)
6	23.5	0.35	10.7; 8.8	3 α , 5, 7, 13, 15	23.6	0.34	6	68.8	4.63
7	25.1	0.74	8.8; 10.7; 5.4	6, 8 α , 13	25.1	0.73	7	52.2 (49.4)	0.81 (0.86)
8 α	20.4	1.86	5.4	7, 8 β	20.5	1.84	8	25.1 (30.1)	1.25 (1.33)
8 β		1.23	10.7	5, 8 α , 12		1.22	8		
9 α	39.4	1.88		9 β	39.3	1.88	9	24.3	2.08
9 β		1.71		1, 8 β , 9 α , 14		1.71	9		2.32
10	74.6				74.7		10	138.9	
11	19.3				19.2		11	32.2 (31.7)	1.63 (1.63)
12	15.5	1.00		5, 8 β	15.6	0.99	12	21.12	1.00
13	28.8	1.05		6, 7	28.8	1.05	13	21.12	1.00
14	30.7	1.16		1, 2 α , 5, 9 α	30.7	1.15	14	16.9 (22.0)	1.56 (1.61)
15	16.1	0.95		3 α , 4, 6	16.1	0.94	15	16.4	1.46

^a Chemical shifts relative to CHCl₃ (7.27 ppm for ¹H) and CDCl₃ (77 ppm for ¹³C). ^b Values in parentheses represent the shifts for the second kunzealol conformer.

tuberosum is reported here. The previously unknown potato sesquiterpene alcohols (abbreviations according to a previous paper by Szafranek (5)), namely, I and III, were obtained by steam-distillation, preparative column chromatography, and separation into fractions by HPLC on a silica gel column. The HPLC-separated fractions were then analyzed by GC-FID, GC-MS, and NMR. To verify whether the alcohols obtained by steam-distillation and liquid chromatography separation were really potato compounds, GC-FID and GC-MS analyses of a methylene chloride extract of potato leaf surfaces were carried out. The two HPLC-separated fractions (**Figure 3**) contained artifacts arising from steam-distillation. Although this process entails the risk of sesquiterpene alcohol degradation (12), it is a necessary step in the isolation of sesquiterpenes from the substances present in potato leaf waxes, mostly long-chain hydrocarbons.

We developed an HPLC-LSD method for separating the sesquiterpene alcohols (**Figure 3**). This separation resulted in clearly resolved peaks corresponding to four compounds. GC-MS analyses showed the compounds to be 95–99% pure. This method offered a readily evaporated organic mobile phase and good reproducibility of separation. HPLC is often used for isolating compounds from essential oils for further GC-MS and NMR analysis (13). Unfortunately, many sesquiterpenoids lack chromophoric groups, so the use of this method is often limited to low UV monitoring (220 nm). A light-scattering detector (a semi-universal detector) can detect all compounds that cannot evaporate (14). The separation of volatile sesquiterpene alcohols is easily achieved because of the possibility of working with solvent that is more volatile than the analyte.

Sesquiterpene alcohol I (RI 1600 on RTX-5 liquid phase) was identified as ledol (**Figure 1**). According to HR mass spectrometry measurements, the exact mass was 222.198, which corresponds to an elemental composition of C₁₅H₂₆O with three indices of unsaturation. Since no double bond was found in the ¹³C NMR spectrum (**Table 1**), all three unsaturations are rings. **Figure 2** shows the mass spectrum of potato sesquiterpene alcohol I; it was identical to that of the sample directly solvent-extracted from the potato leaves, which rules out the possibility of degradation during the steam-distillation step. Moreover, it

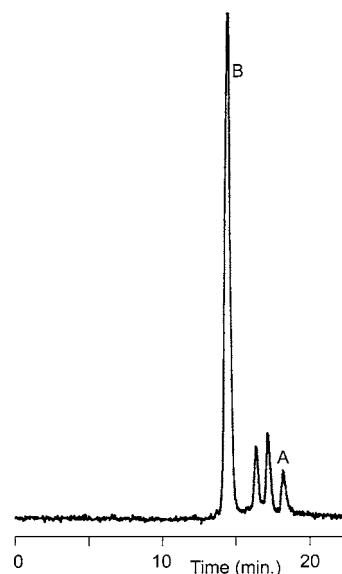


Figure 3. HPLC-LSD chromatogram of potato leaf sesquiterpene alcohols from the Mila variety. Peak A, ledol; peak B, kunzealol. The remaining two peaks are artifacts arising from the steam-distillation.

was identical to the mass spectrum of the ledol standard separated from *L. palustre*. The general appearance of the mass spectrum resembled the literature spectra of ledol or globulol (6), but the literature retention indices on the RTX-5 liquid phase are RI = 1565 and 1583, respectively (6). In the present study, however, ledol separated from potato leaves and the ledol standard separated from *L. palustre* exhibited a different RI (=1600) and was eluted after germacrene D-4-ol, the other potato sesquiterpene alcohol, (RI = 1573 in our study and 1574 according to literature data (6)). In our opinion, the literature data (6) regarding the RI of ledol is inaccurate. The identification of potato ledol was additionally confirmed by co-injection with the ledol standard on RTX-5 and SolGel-I liquid phases. Weissbecker et al. (3) also suggested that ledol is a component of potato volatiles with an RI of 1605, but the identification was based only on GC and GC-MS studies.

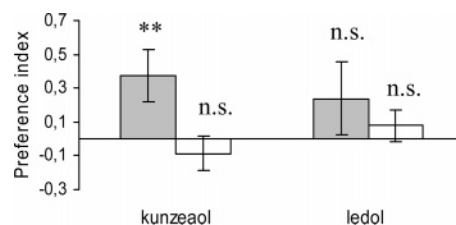
Table 2. Proton–Carbon Connectivity Assigned from the HMBC Experiments for Ledol and Kunzeaol

ledol H–C → C	kunzeaol H–C → C
3 α → 3	12 → 7, 11, 13
6 → 13	13 → 7, 11, 12
8 α → 7	15 → 3, 5
9 α → 10	
12 → 6, 7, 11, 13	
13 → 6, 7, 11, 12	
14 → 1, 9, 10	
15 → 3, 4, 5	

¹H and ¹³C NMR spectra of sesquiterpene alcohol I are complex systems displaying several overlapping resonances. Two-dimensional experiments such as DQF-COSY, HSQC, and HMBC were used for assigning signals. The observed resonances are summarized in **Tables 1** and **2**. The NMR data (¹H and ¹³C) of sesquiterpene alcohol I are virtually identical to that of literature data (8, 11) and of the ledol standard separated from *L. palustre*. Furthermore, the spectra differed from those of viridiflorol, epiglobulol, and globulol (11).

The skeleton structure of sesquiterpene alcohol I is obviously *allo*-aromadendran-10-ol. The presence of a three-membered ring in the compound is evident from the HMBC experiment, where four correlations are found for two methyl groups (12 and 13) as entry windows (**Table 2**). Clearly, these are *gem*-methyls attached to the quaternary carbon (¹³C: δ 19.3) of the three-membered ring. The stereochemistry of the compound was assigned from nuclear Overhauser effects (NOE) (**Table 1**) compared with literature data (8, 11). The H-14 showed a strong NOE interaction with H-1 and a weak interaction with H-5. The identification of ledol was additionally confirmed by the chemical shifts of the cyclopropane ring protons (δ 0.35 and 0.74), which are reported to be different for other stereoisomers, viridiflorol and globulol (8).

Sesquiterpene alcohol III (RI 1688 on RTX-5 liquid phase) was identified as 6- α -hydroxygermacra-1(10),4-diene (kunzeaol) (**Figure 1**). The HPLC-separated sample was found to be homogeneous by capillary GC, and its mass spectrum was identical to that from the solvent extract of the potato leaf surface. The fragmentation pattern of the mass spectrum of sesquiterpene alcohol III obtained from the potato (**Figure 2**) was very similar to that of the kunzeaol from *Kunzea* identified by Cornwell et al. (15). Interestingly, the ion with *m/z* 84 and composition C₅H₈O⁺ forms the base peak in the spectrum, which is quite unusual in sesquiterpenes. Again, the molecular ion appeared at *m/z* 222.198 in HR mass spectrometry measurements, which corresponds to an elemental composition of C₁₅H₂₆O with three indices of unsaturation. The ¹³C NMR spectrum (**Table 1**), in turn, showed only four resonances (for one conformer) in the range between 120 and 135 ppm, which suggests that two of the above-mentioned three unsaturations correspond to double bonds and that the third one must be a ring. The HMBC (**Table 2**), HSQC, and COSY experiments helped significantly in assigning the signals. The ¹H NMR spectrum displayed broad peaks. All the ¹H and ¹³C data of potato sesquiterpene alcohol III (**Table 1**) are consistent with those published by Cornwell et al. (15) for kunzeaol. Furthermore, the NMR data (**Table 1**) revealed two sets of resonances of different intensities as a result of the compound being a mixture of two conformers, similar to a previous report (15). To our knowledge, kunzeaol has previously been reported only in *Kunzea* species (Myrtaceae) (15) and in *Origanum* (Lamiaceae) (16); this is the first report of the presence of kunzeaol

**Figure 4.** Preference indices (mean \pm SE, $N = 15$) for CPB adults (full bars) and CPB larvae (empty bars) feeding on the potato leaf discs treated with kunzeaol (1.2 μ g on 2.1 cm² disc) or ledol (0.3 μ g on 2.1 cm² disc). Comparison of consumed areas on test and control discs: **, significant; $P < 0.01$; n.s., not significant (Wilcoxon test).

in Solanaceae. Potatoes also produce another sesquiterpene alcohol with the structure of 4- β -hydroxygermacra-1(10),5-diene (germacrene D-4-ol) (5), just as *Kunzea* plants do (15).

Bioassays of Potato Sesquiterpene Alcohols. We examined the influence of both potato-derived sesquiterpene alcohols, ledol and kunzeaol, on the feeding behavior of the CPB adults and larvae in a choice bioassay. The activity of the compounds was tested at concentrations about five times greater than those occurring naturally on potato leaf surfaces. A preference index was used to compare the influence of sesquiterpene alcohols on the feeding behavior of the insect (**Figure 4**). Kunzeaol showed significant (Wilcoxon test, $P < 0.01$) phagostimulatory activity (PI = 0.38) in tests with CPB adults at that concentration.

L. palustre is traditionally used in rural Poland to protect woolen clothes from moths. Since this repellent plant contains ledol, it was interesting to study the influence of ledol on CPB behavior. Unfortunately, however, ledol did not affect significantly (Wilcoxon test, $P > 0.05$) the feeding behavior of either larval or adult CPB.

The kunzeaol content (sesquiterpene alcohol III) in plant leaves varies among the potato varieties (5). *S. tuberosum* cv. Mila produces significant amounts of this attractant and can be distinguished from other potato varieties containing moderate levels of sesquiterpene alcohols. Large aggregations of CPB have been observed on the Mila variety (17), which could confirm its attractiveness to CPB adults. A potato variety containing significant amounts of the CPB feeding attractant kunzeaol could be used as a trap crop. Additionally, the observed differences in kunzeaol contents among potato varieties can be used in breeding for potatoes with CPB resistance. Current pest management practice relies mostly on pesticides, but greater plant resistance could reduce pesticide use.

ACKNOWLEDGMENT

We are grateful to the Herbagol, Białystok, Poland, for supplying *Ledum palustre*.

LITERATURE CITED

- Bolter, C. J.; Dicke, M.; Van Loon, J. J. A.; Visser, J. H.; Posthumus, M. A. Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *J. Chem. Ecol.* **1997**, *23*, 1003–1023.
- Weissbecker, B.; Schütz, S.; Klein, A.; Hummel, H. E. Analysis of volatiles emitted by potato plants by means of a Colorado beetle electroantennographic detector. *Talanta* **1997**, *44*, 2217–2224.

- (3) Weissbecker, B.; Van Loon, J. J. A.; Posthumus, M. A.; Bouwmeester, H. J.; Dicke, M. Identification of volatile potato sesquiterpenoids and their olfactory detection by the two-spotted stinkbug *Perillus bioculatus*. *J. Chem. Ecol.* **2000**, *26*, 1433–1445.
- (4) Dickens, J. C. Orientation of Colorado potato beetle to natural and synthetic blends of volatiles emitted by potato plants. *Agric. Forest Entomol.* **2000**, *2*, 167–172.
- (5) Szafranek, B.; Chrapkowska, K.; Pawińska, M.; Szafranek, J. Analysis of leaf surface sesquiterpenes in potato varieties. *J. Agric. Food Chem.* **2005**, *53*, 2817–2822.
- (6) Adams, R. P. *Identification of Essential Oils Components Gas Chromatography/Mass Spectrometry*; Allured Publishing: Carol Stream, IL, 1995.
- (7) Grange, A. H.; Sovocool, G. W. Identification of unanticipated compounds by high resolution mass spectrometry. *Spectroscopy* **2003**, *18*, 12–24.
- (8) Kaplan, M. A. C.; Pugialli, H. R. L.; Lopes, D.; Gottlieb, H. E. The stereochemistry of ledol from *Renealmia chrysotrycha*: An NMR study. *Phytochemistry* **2000**, *55*, 749–753.
- (9) Kir'yalov, N. P. Principal components of the essential oil of *Ledum palustre*. *Dokl. Akad. Nauk S.S.S.R.* **1948**, *61*, 305–308.
- (10) Pletnev, V. Z.; Tsygannik, I. N.; Fonarev, Yu. D.; Mikhailova, I. Yu.; Miroshnikov, A. I. Molecular structure of ledol revealed by X-ray analysis. *Bioorg. Khim.* **1993**, *19*, 366–372.
- (11) Bombarda, I.; Raharivelomanana, P.; Ramanoelina, P. A. R.; Faure, R.; Bianchini, J.-R.; Gaydou, E. M. Spectrometric identifications of sesquiterpene alcohols from niaouli (*Melaleuca quinquenervia*) essential oil. *Anal. Chim. Acta* **2001**, *447*, 113–123.
- (12) Luque de Castro, M. D.; Jimenez-Carmona, M. M.; Fernandez-Perez, V. Towards more rational techniques for the isolation of valuable essential oils from plants. *Trends Anal. Chem.* **1999**, *18*, 708–716.
- (13) Merfort, I. Review of the analytical techniques for sesquiterpenes and sesquiterpene lactones. *J. Chromatogr., A* **2002**, *967*, 115–130.
- (14) Stolyhwo, A.; Colin, H.; Martin, M.; Guiochon, G. Study of the qualitative and quantitative properties of the light-scattering detector. *J. Chromatogr.* **1984**, *288*, 253–275.
- (15) Cornwell, C. P.; Reddy, N.; Leach, D. N.; Wyllie, S. G. Germacradienols in the essential oils of the Myrtaceae. *Flavour Fragrance J.* **2001**, *16*, 263–273.
- (16) Dudai, N.; Larkov, O.; Chaimovitch, D.; Lewinsohn, E.; Freiman, L.; Ravid, U. Essential oil compounds of *Origanum dayi* Post. *Flavour Fragrance J.* **2003**, *18*, 334–337.
- (17) Widerski, K.; Kamasa, J.; Grala, B. The occurrence of Colorado potato beetle on potato varieties and strains. *Materiały XXXIV Sesji Naukowej Instytutu Ochrony Roślin* **1994**, *2*, 56–60.

Received for review June 5, 2006. Revised manuscript received August 11, 2006. Accepted August 11, 2006. The work was partly financed by Grant PBZ-KBN-060/T09/2001/11 from the Polish State Committee for Scientific Research and by Grant BW 8000-5-0413-6 from the University of Gdańsk.

JF0615653