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Received for review January 18, 1989. Accepted May 31, 1989.

Isolation and Characterization of Pentacyclic Triterpene Ovipositional Stimulant for the Sweet Potato Weevil from *Ipomoea batatas* (L.) Lam.

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A methylene chloride extract from the surface of sweet potato storage roots (cultivar Centennial) was fractionated on silicic acid and eluted with hexane, 1:3 methylene chloride–hexane, and 1:1 methylene chloride–hexane. The 1:3 methylene chloride–hexane fraction yielded compound I: >96% purity, the most dominant compound present; mp 238–239.5 °C; IR, 1730, 1250 cm^{-1}. The mass spectrum of compound I displayed a molecular ion at m/z 468 with a molecular formula of $C_{32}H_{52}O_2$. The fragmentation pattern was indicative of a pentacyclic triterpene with isopropyl and acetate moieties. Compound II, which could be produced by the hydrolysis of compound I, was present in the 1:1 methylene chloride–hexane fraction: mp 214–215.5 °C; IR, 3400 cm^{-1}; molecular ion, m/z 426; molecular formula, $C_{30}H_{50}O$. The alcohol of compound I (compound II) was identified as boehmerol. Based on ^{13}C NMR and GC–MS data and physical evidence, the structure of compound I was established and tentatively named boehmeryl acetate. Boehmeryl acetate extracted from the surface of sweet potato storage roots appears to act as an ovipositional stimulant for the sweet potato weevil, *Cylas formicarius elegantulus* Summers.

Sweet potato [*Ipomoea batatas* (L.) Lam.] is a major international staple crop, grown extensively throughout the tropical and temperate zones for its edible storage roots (Pardales and Cerna, 1987). A constraint to sweet potato production in both tropical and temperature growing areas is the sweet potato weevil, *Cylas spp.* (Edmonds, 1971; Schalk and Jones, 1985), which feeds on all parts of the plant and lays its eggs in the storage roots (Reinhard, 1923; Cockerham et al., 1954). The development of insect-resistant sweet potato lines is seen as an essential component in the management of this pest (Martin and Jones, 1986). Son et al. (1989) demonstrated that there are significant differences in surface components of sweet potato storage roots between susceptible lines and those displaying a moderate level of resistance to the weevil [resistance estimates based on field evaluation (Mullen et al., 1981; Mullen et al., 1985)]. Wilson et al. (1988) demonstrated that a methylene chloride surface extract of the periderm of storage roots of the susceptible line Centennial stimulated oviposition of the weevil, *Cylas formicarius elegantulus* (Summers). Nottingham et al. (1987) also showed that ovipositional stimulant resided in the root periderm, not in the core of the storage root. In addition, it has been established that the major component (compound I) of the surface extract was an ovipositional stimulant of female weevils (Wilson et al., 1989).

In this paper, the isolation of two pentacyclic triterpenoids from the storage root of Centennial and the spectral evidence leading to the elucidation of their structures are presented.

MATERIALS AND METHODS

Materials. Sweet potato cultivar Centennial was grown at the University of Georgia Horticulture Farm during 1986 and 1987. After harvesting, the storage roots were washed, air-dried, and cured for 7 days at 29 °C and 90% RH and stored at 13 °C and 85% RH. Storage roots used for analysis were

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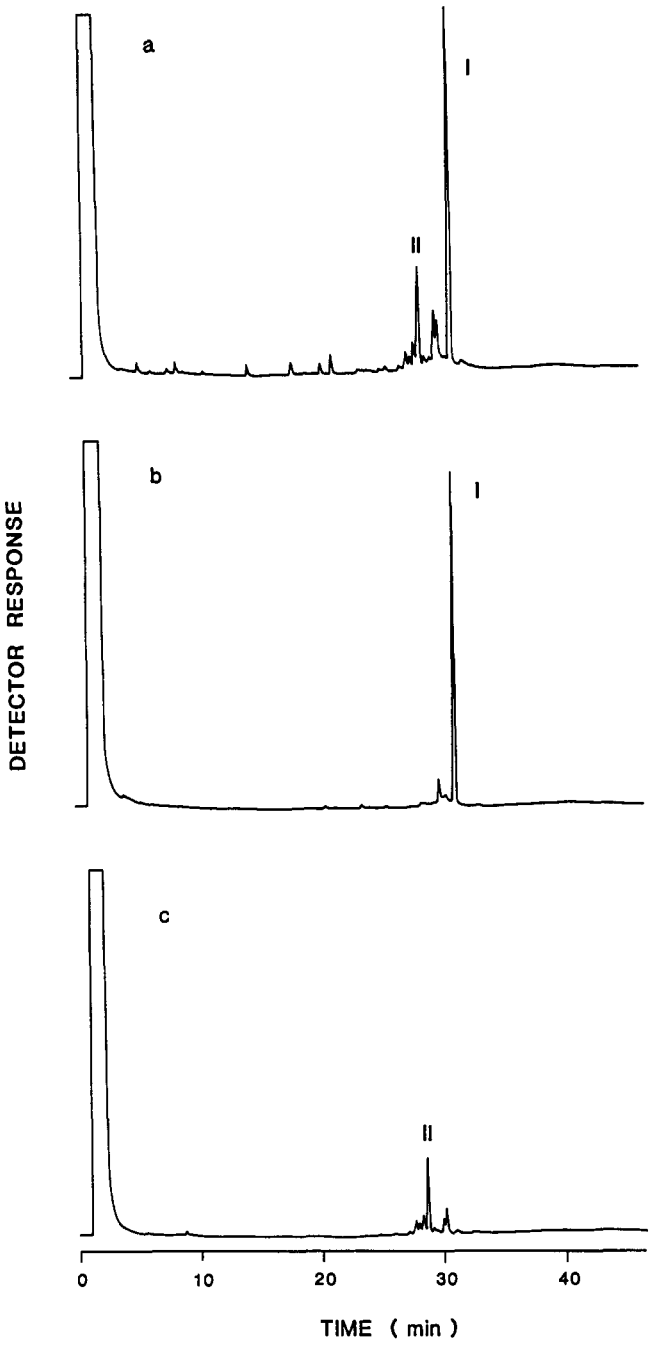

Figure 1. Capillary gas chromatograms of silylated root surface fraction from Centennial: (a) crude extract; (b) silicic chromatographic fraction 2; (c) silicic acid chromatographic fraction 3.

U.S. No. 1 grade, having surface areas of 120–150 cm^2 free of physical damage.

Solvents (distilled-in-glass grade) used were from Burdick and Jackson Laboratory Inc., Muskegon, MI. (Trimethylsilyl)imidazole (TMSI) and Tri Sil-Z were obtained from Pierce Chemical Co. (Rockford, IL). Unisil silicic acid was obtained from Clarkson Chemical Co., Williams Port, PN.

Gas Chromatographic Analyses. An amount of extract equivalent to 350 μg was transferred to a microautosampler vial and the solvent removed under nitrogen at 40 °C. Sixty microliters of 3:1 Tri Sil-Z–TMSI derivatizing reagent was added and the microautosampler vial sealed with crimp cap. After being heated for 45 min at 76 °C, the samples were analyzed on a Hewlett-Packard 5700A gas chromatograph [equipped with a 7672A autosampler and modified for GC-2 as described by Severson et al. (1982)] using a 0.3 mm (i.d.) × 30 m thin film (about 0.1 μm) SE-54 fused silica column (Arrendale et al., 1984) (temperature program, 150–290 °C at 4 °C/min, 15 min hold at 290 °C; flow rate, 54 cm s^{-1} H_2; split flow rate, 46 mL min^{-1} H_2; injection port temperature, 250 °C; flame ionization detector temperature, 300 °C).

Table I. ^{13}C NMR Spectral Data (Chemical Shifts) of Compound I, Compound II, and Boehmerol

C	I (acetyl)	II (hydroxyl)	boehmerol
1	32.9	33.2	33.4
2	25.3	20.2	20.3
3	82.0	79.3	79.3
4	38.2	39.1	39.2
5	46.3	46.2	46.5
6	22.6	22.6	22.7
7	30.4	30.4	30.5
8	41.4	41.4	41.5
9	48.2	48.1	48.2
10	37.1	37.1	37.2
11	18.8	18.9	19.0
12	26.4	26.4	26.4
13	131.0	131.2	130.8
14	42.5	52.5	52.6
15	26.4	26.5	26.5
16	34.8	35.0	35.0
17	42.8	42.8	42.7
17	141.8	141.7	142.0
19	27.6	27.6	27.5
20	37.5	37.5	37.6
21	58.6	59.1	59.2
22	29.8	29.8	29.8
23	28.9	29.0	29.1
24	17.2	16.1	16.1
25	17.9	17.9	18.0
26	23.0	23.1	23.0
27	26.7	26.7	26.7
28	25.7	25.8	25.7
29	22.9	22.9	23.0
30	22.9	22.9	23.0
>C=O	171.0		
CH_3	21.3		

Extraction and Isolation. Four to five storage roots at a time were placed in a 4-L beaker containing 2 L of methylene chloride, and the mixture was ultrasonically extracted for 8 min (Son et al., 1989) to yield about 450 mg of extract from 12 kg of storage roots. Extracts were stored at −18 °C.

Extract (100 mg in hexane) was placed on a Unisil silicic acid column (10 g, operated at 5 psi under N_2) and eluted with 100 mL of hexane (fraction 1), 500 mL of 1:3 methylene chloride–hexane (fraction 2), and 500 mL of 1:1 methylene chloride–hexane (fraction 3). The major GC volatile component (96+%, compound I) in the surface extract (Figure 1a) eluted in fraction 2 (Figure 1b) was recrystallized from hexane to yield a white solid: 99+%; mp 238–239.5 °C; IR, 1730, 1250 cm^{-1}; MS, *m/e* 468). A 30-mg portion of fraction 2 was hydrolyzed with 1 N KOH in 85% EtOH in water (50 mL) by heating at 76 °C for 4 h. After cooling, 20 mL of H_2O was added, the mixture was partitioned with hexane, and the hexane solubles were recrystallized to yield white crystals of II: 99+% by GC; mp 214–215.5 °C; IR, 3400 cm^{-1}; MS, *m/e* 426. Compound II was the major component in fraction 3 (Figure 1c). About 100 μg of II in 40 μL of methylene chloride was converted to I by treatment for 24 h with 60 μL of acetic anhydride and 5 μg of 4-(dimethylamino)pyridine.

Spectral Analysis. Capillary GC–MS data and direct-insertion probe mass (electron impact, 70 eV) were obtained on an HP 5985 as modified by Arrendale et al. (1984). IR spectra were obtained as KBr disks on a Perkin-Elmer Model 684 spectrophotometer. ^1H and ^{13}C NMR data were obtained in 100% $CDCl_3$ with TMS as the internal standard with a Bruker AM-250 FT NMR spectrometer operating at 25 °C.

RESULTS AND DISCUSSION

The gas chromatogram (Figure 1a) of the silylated root extract contained two major components (components I and II). Several silylation reagents, i.e., BSTFA [*N,O*-bis(trimethylsilyl)trifluoroacetamide], BSA [*N,O*-bis(trimethylsilyl)acetamide], and TMSI (trimethylsilyl)imida-

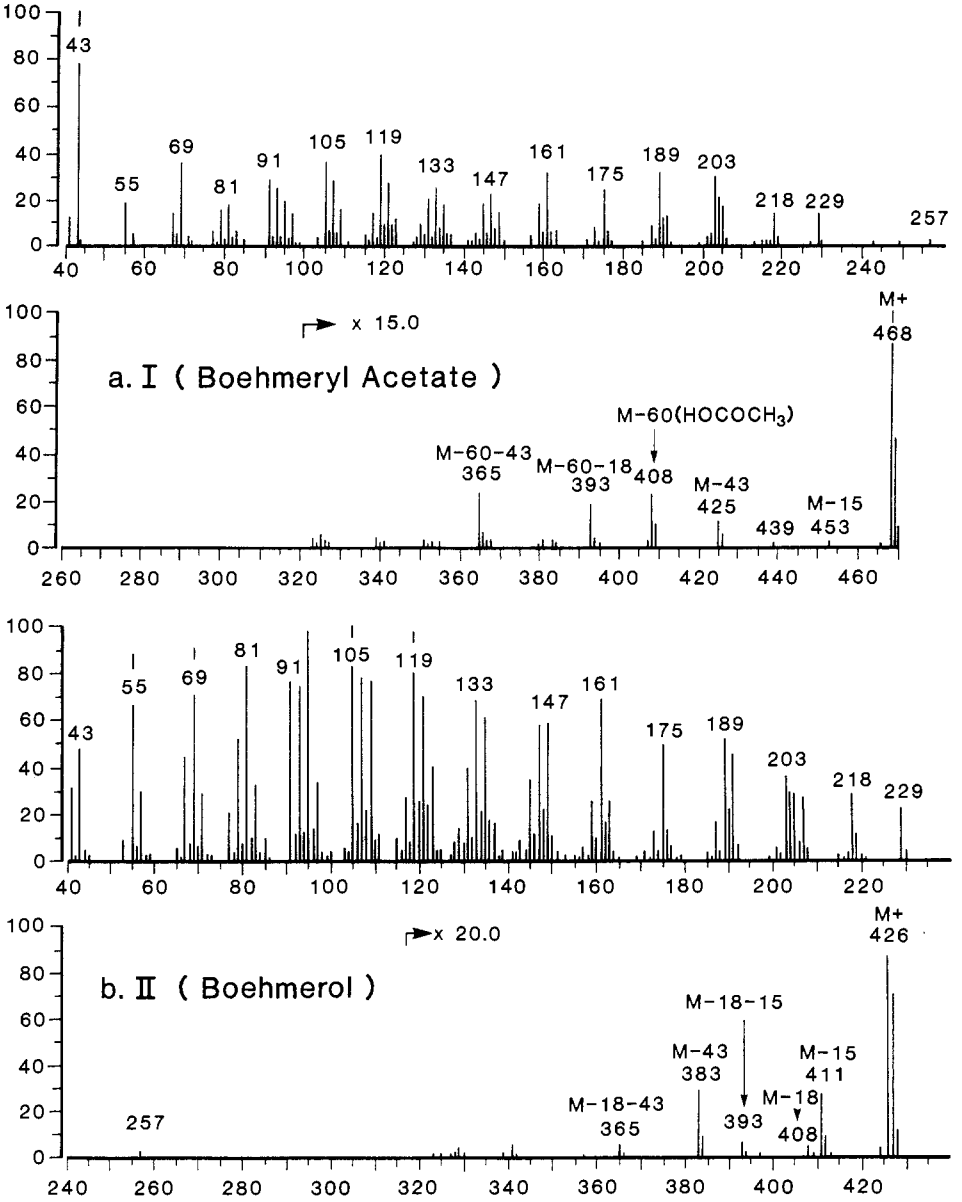

Figure 2. Mass spectra of compound I (a) and compound II (b) by direct-insertion probe (70 eV).

zole–pyridine, were investigated. TMSI–pyridine reagent mixture was the only system that quantitatively converted II to its silyl ether. The major component extracted from the weevil-susceptible cultivar Centennial, compound I, represented 50% of the GC-volatile material (Figure 1b). The silicic acid fraction 2 yield 95+% I, which was recrystallized from hexane in 99+% purity; mp 238–239.5 °C. IR data indicated strong absorption at 1250 and 1730 cm^{-1}. Treatment of I with the derivatizing agent did not change its GC retention or mass spectrum.

The mass spectrum of I (Figure 2a) indicated a molecular ion at m/z 468. Fragment ions at m/z 408 (M – 60) and 43 indicated the presence of acetyl ester and 453 (M – 15), 425 (M – 43), 393 (M – 60 – 15), and 365 (M – 60 – 43) indicated the presence of an isopropyl group. Several other mass fragments (e.g., m/z 189, 203, 218) suggested that I was either a hopane or lupane type of pentacyclic triterpenoid (Budzikiewicz et al., 1963). The absence of the m/z 73 peak, characteristic of a trimethylsilyl ester or ether, indicated that I did not contain a free hydroxyl or acid moiety. Two olefinic carbon singlets at 141.8 (C-18) and 131.3 (C-13) in the ^{13}C NMR spectrum and the presence of a single proton (4.48 ppm) above 2.4 ppm in the ^{1}H spectrum indicated one tetrasubstituted double bond. The ^{13}C NMR showed the presence of 32 carbons atoms (Table I) and confirmed the presence of a carbonyl group as a singlet at 171.0 ppm (Table I). From these data the formula $C_{32}H_{52}O_2$ was deduced.

Recrystallization of the hydrolysis product of I produced compound II (mp 214–215.5 °C) with an apparent molecular ion at 426 (Figure 2b). The mass spectrum of the trimethylsilyl derivative of compound II had a molecular ion at m/z 498 and major high-mass ions at m/z 483 (M – 15) and 408 (M – OH TMS) (data not shown), indicative of a monosilyl ether. Acetylation of II produced a product identical with compound I. GC retention and GC–MS data showed compound II was the major component in silicic acid fraction 3 (Figure 1c).

The IR spectrum of compound II displayed a strong absorption at 3400 cm^{-1} (OH). The ^{1}H NMR spectrum showed a doublet of a doublet centered at 3.24 ppm characteristic of a proton geminal to the β-hydroxyl group at C-3 where a chemical shift (1.24 ppm upfield) occurred due to replacement of the acetyl moiety with a hydroxyl group. Comparison of the ^{13}C NMR spectrum indicated the disappearance of the 171.0 and 21.3 ppm signals in

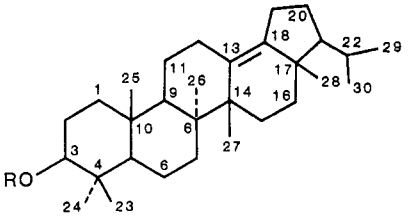

Figure 3. Structures of compound I (boehmeryl acetate, R = CH_3CO) and compound II (boehmerol, R = H).

compound II indicative of >C=O and CH_3 groups, respectively.

On the basis of physical and chemical evidence, the structure of I was determined to be the acetate of boehmerol ($C_{30}H_{50}O$), a pentacyclic triterpenoid recently identified from *Boehmeria excelsa* (Figure 3) (Oyarzun et al., 1987). The resonance for C-2, C-3, C-4, and C-24 in boehmeryl acetate (compound I) were shifted due to the presence of the β-acetate moiety (Table I). ^{13}C NMR spectral data for II coincided precisely with the alcohol boehmerol.

Boehmeryl acetate, a member of a relatively new group of pentacyclic triterpenoids, was isolated from the surface of sweet potato storage roots and was an ovipositional stimulant for the sweet potato weevil, *C. formicarious elegantulus* (Summers) (Wilson et al., 1989). Identification of biologically active compounds that modulate insect behavior represents an important step in developing an analytical approach to breeding for resistance to specific insect pests.

Registry No. I, 106973-34-6; II, 123409-83-6.

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Received for review September 1, 1988. Accepted May 17, 1989.