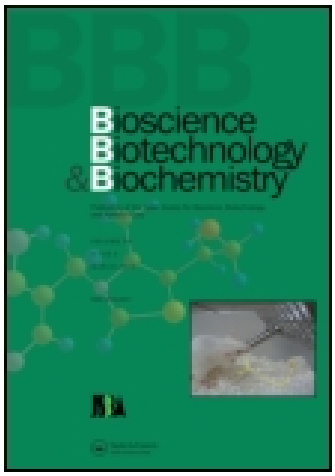

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(*E*)-2-(4′-Methyl-3′-pentenylidene)-4-butanolide, Named β-Acariolide: A New Monoterpene Lactone from the Mold Mite, *Tyrophagus putrescentiae* (Acarina: Acaridae)†

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Reinvestigation of the opisthonotal gland secretion of the mold mite, *Tyrophagus putrescentiae*, resulted in the isolation of a new monoterpene lactone, whose chemical structure was elucidated as (*E*)-2-(4′-methyl-3′-pentenylidene)-4-butanolide (3), to which we gave the trivial name β-acariolide in relation to β-acaridial {1, (*E*)-2-(4-methyl-3-pentenylidene)-butanedial}. The compound was synthesized by $LiAlH_3$ (OEt) reduction of 1 and subsequent oxidation involving simultaneous cyclization by using Ag_2CO_3 on Celite. Both the *E*- and *Z*-isomers of β-acariolide (3 and 4) were also prepared by the reaction of α-ethoxaly-γ-butyrolactone (6) and 4-methyl-3-pentenal under basic conditions. Their NMR spectra were compared with each other, and the geometry of the pentenylidene double bond of the isolated compound was concluded as being *E*.

Key words: (*E*)-2-(4′-methyl-3′-pentenylidene)-4-butanolide; β-acariolide; *Tyrophagus putrescentiae*; opisthonotal gland; Acaridae

A total of about fifty compounds have so far been identified in the opisthonotal gland secretion from 31 species belonging to seven families of astigmatid mites. Most of their biological functions remain obscure, although several function as semichemicals such as alarm, aggregation, and sex pheromones, and also as antifungal compounds.[1] Six new monoterpenes[2,3] and three new salicylaldehyde analogs[4,5] have also been found in the secretion, among which four compounds were antifungal.[5,6]

We reexamined the gland exudate of *Tyrophagus putrescentiae* SCHRANK and found that, apart from the already reported neryl formate,[7] citral,[8] 2-hydroxy-6-methylbenzaldehyde,[9] β-acaridial {**1**, 2(*E*)-(4-methyl-3-pentenylidene)-butanedial},[3] hexyl linolate,[10] and hydrocarbons,[11] the gland also contained a novel monoterpene lactone, whose structural elucidation and synthesis we report here.

The hexane extract (500 mg), obtained by soaking mites (100 g) for 3 min, showed a small but noteworthy peak by GLC at 9.11 min, corresponding to 5.3% of the total area. This peak was recovered in a fraction (33 mg) of the hexane–ether (4 : 1) eluate from an SiO_2 column. The fraction was further purified in the SiO_2 column and by preparative TLC (hexane–ether (1 : 1) as the developing solvent) until it gave one single peak from the CP-Sil column and one UV (254 nm)-positive spot by TLC (R_f, 0.25 under the same conditions as those just stated).

The MS spectrum of the isolated pure compound (**3**, 2.3 mg) revealed the M^+ ion at *m/z* 166.0977 ($C_{10}H_{14}O_2$, calcd. as 166.0992) by precise mass spectrometry, and the resulting molecular formula was the same as that of β-acaridial (**1**). Although the intensities were different, the MS spectrum (Fig. 1) consisted of almost the same fragments as those of **1**, as reported.[3] The fragment ion ($M^+ - 43$) due to the unusual cleavage with double bond migration was observable at *m/z* 123 as a major fragment, while the ion at *m/z* 137 due to the loss of –CHO from **1** was missing. This indicates that compound **3** was neither aldehydic nor alcoholic. Treatment of **3** with $LiAlH_3$ (OEt) gave an alcohol, whose mass spectrum and GC retention time were both identical to those of β-acaridiol (**2**) derived from **1**.

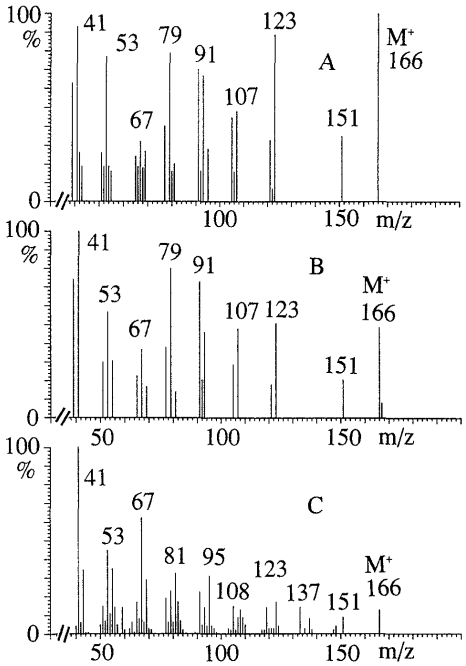

Fig. 1. Mass Spectra of A) Natural β-Acariolide (**3**), B) Synthetic β-Acariolide (**3**), and C) Standard β-Acaridial (**1**).

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The unusually higher C=O band at 1797 cm^{-1} by GC-FTIR, together with C–O bands at 1195 and 1041 cm^{-1} (Fig. 2), suggested the presence of a five-membered lactone in the molecule of **3**. A conjugated C=O band was also observed at 1681 cm^{-1}. The ^{13}C-NMR spectrum (125.8 MHz, $CDCl_3$) gave all the chemical shifts corresponding to ten carbons. The chemical shift at 171.4 ppm (C=O, s), together with the one at 65.4 ppm (–CH_2–O–, t), indicated the lactone moiety in **3**. A total of four sp^2 carbons at 118.8 ppm (d), 124.8 (s), 134.6 (s), and 139.4 (d) corresponded to two double bonds. The other four carbons were observed at 29.2 ppm (t), 25.6 (q), 25.1 (t), and 17.9 (q). This information coupled with mass spectral and GC-FTIR data suggested that the structure of **3** possessed a lactone ring with two double bonds. ^{1}H-NMR suggested all 14 protons in the molecule. Compound **3** gave a UV-positive spot by TLC as already mentioned; therefore, a conjugated moiety must have been incorporated either between the double bonds or between a double bond and a carbonyl group. ^{1}H-NMR together with ^{13}C-NMR demonstrated the presence of a prenyl group; two methyls at δ 1.72 (3H, s) and 1.65 (3H, s), a vinyl proton at δ 5.13 (1H, tq, J=7.2 Hz and 1.4 Hz) and two methylene protons at δ 2.88 (4H, br. t), together with a substituted sp^2 carbon at 134.6 ppm (s). All of the other protons observed were a vinyl at δ 6.71 (1H, m), and an oxygen-substituted methylene at δ 4.39 (2H, t, J=7.3 Hz). These observations suggested that the lactone carbonyl must have been conjugated to the other double bond (–CH=C<) exocyclically in the molecule. From these data, we postulate the structure of this monoterpene lactone (**3**) as 2-(4′-methyl-3′-pentenylidene)-4-butanolide. The configuration of the $C_{1'}=C_2$ double bond remained to be determined, although *E*-geometry seemed to be self-evident because the lactone was retro-synthetically deducible as an oxidation and cyclization product of β-acaridiol (**2**), whose configuration was *E*.

E-Lactone **3** was synthesized by Celite-Ag_2CO_3 oxidation[12] involving simultaneous cyclization, starting from **2** which was prepared by $LiAlH_3$ (OEt) reduction of **1** (Fig. 3).[3] The reaction product gave a single spot by TLC and an identical t_R value by GLC co-injection to that of the isolated compound. MS (Fig. 1) and NMR data (stated above) of the isolated natural compound were also identical to those of the synthetic *E*-lactone.

In order to confirm the configuration of the $C_{1'}=C_2$ double bond, both isomers of the *E*- and *Z*-lactones (**3** and **4**) were prepared according to the reported method[13] by reacting 4-methyl-3-pentenal with α-ethoxaly-γ-butyrolactone (**6**). Compound **6** was obtained from the reaction of γ-butyrolactone (**5**) and diethyl oxalate under basic conditions as reported,[14] and 4-methyl-3-pentenal was used without purification after acid hydrolysis (80% acetic acid in water at r.t. for 3 h) of 4-methyl-3-pentenal diethyl acetal. The resulting product mixture was separated to each geometrical isomer (**3** and **4**) by HPLC in an ODS column.

The geometry of each isomer was determined from the following evidences: the 1′-proton in **3** appeared at δ 6.69, while the one in **4** was at δ 6.16. The relationship between these chemical shifts was identical to that of corresponding protons in both isomers of methyl 2-methyl-2-butenoate (*E*, 6.73; *Z*, 5.98). This means that *E*-geometry should be assigned to **3** and *Z*-geometry to **4**. The corresponding proton of the isolated natural lactone appeared at δ 6.71, and therefore, *E*-2-(4′-methyl-3′-pentenylidene)-4-butanolide (**3**) was concluded as being the structure of the compound. The chemical shifts of the 2′-methylene also differed between the two isomers (*E*, δ 2.88; *Z*, 3.42), and the chemical shift of the 2′-proton of the isolated lactone (**3**) was the same as that of the synthetic *E*-isomer (δ 2.88).

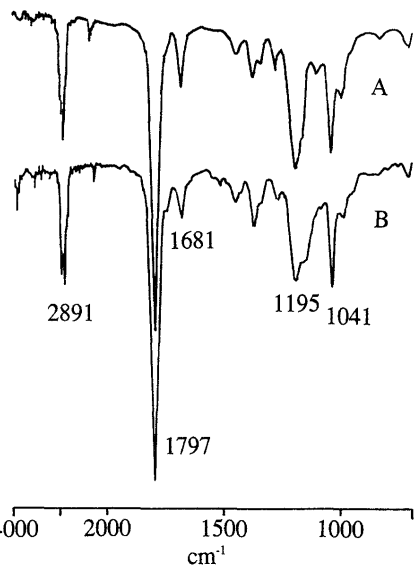

Fig. 2. GC-FTIR of A) Natural β-Acariolide (**3**) and B) Synthetic β-Acariolide.

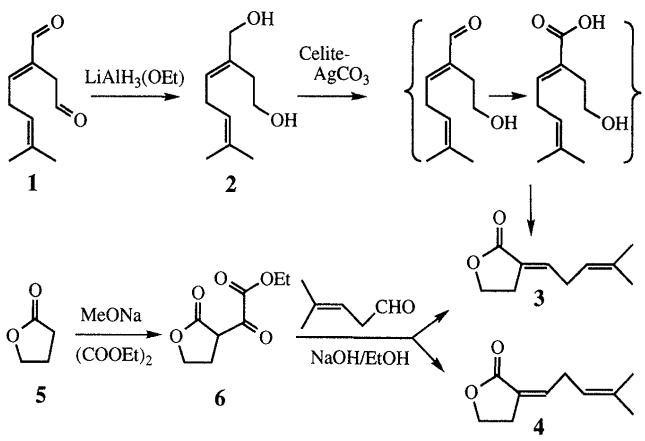

Fig. 3. Synthetic Route to β-Acariolide (**3**) and Its *Z*-Isomer (**4**).

Based on the above-mentioned data, the natural lactone was identified as **3**, which has not been previously reported from either synthetic nor natural sources. We suggest, therefore, the trivial name β-acariolide (**3**) for the lactone, because β-acaridial (**1**) may be a possible precursor of the biosynthesis. The semiochemical function of lactone **3** for the mold mite is obscure at the moment.

Experimental

Material and methods. Mites were separated from the culture medium and extracted as reported.[14] GLC was conducted in a Hitachi 263-30 instrument with a CP-Sil 19CB capillary column (0.22 mm × 25 m, Chrompack) at a temperature programmed from 135 to 250° at 4°C/min. LC was conducted with Wako-gel C-200, and silica gel 60 HF_{254} (E. Merck, 5 cm × 20 cm, 0.25 mm in thickness) was used for preparative and analytical TLC. NMR data were measured by a Bruker 500 MHz instrument and a JEOL FX-100 NMR spectrometer. IR was obtained by a Shimadzu GC-FTIR instrument, using a wide-bore PEG-20M column. MS were

measured by a Hitachi M-80B high-resolution mass spectrometer.

Synthesis (E)-2-(4'-methyl-3'-pentenylidene)-4-butanolide (**3**) *from β-acaridial* (**1**).

Preparation of β-acaridiol (**2**). β-Acaridial (**1**, 9 mg) isolated from the mold mite (100 g)[3] was reduced by $LiAlH_3$ (OEt) as reported[3] to give **2** (4 mg, 43% yield). ^1H- and ^{13}C-NMR data were identical to those reported.[3]

(E)-2-(4'-methyl-3'-pentenylidene)-4-butanolide (**3**) *from* **2**. β-Acaridiol (**2**, 4 mg) was refluxed with Celite–Ag_2CO_3 (0.57 g) in benzene for 17 h as reported.[12] After removing the solid matter by filtration, the concentrated filtrate was chromatographed in an SiO_2 column to give compound **3** (3.6 mg, 92% yield). Its spectral data were identical to those of the natural product.

Preparation of (E)- and (Z)-2-(4'-methyl-3'-pentenylidene)-4-butanolide (**3** *and* **4**) *from γ-butyrolactone* (**5**).

α-Ethoxalyl-γ-butyrolactone (**6**). Diethyl oxalate (11 g) was reacted with γ-butyrolactone (**5**, 6.5 g) as reported[13] to give **6** (10 g, 72% yield). MS *m/z* (%): 41 (41), 55 (18), 69 (12), 86 (23), 113 (100), and 186 (7); ^1H-NMR (500 MHz) δ_H: 10.91 (1H, bs), 4.49 (2H, t, 7.6 Hz), 4.37 (2H, q, 7.1 Hz), 3.29 (2H, t, 7.6 Hz), 1.39 (2H, t, 7.1 Hz); ^{13}C-NMR (125.8 MHz) δ_C: 176.5 (–C=O), 162.0 (–C=O), 151.8 (–C=), 105.5 (–C=), 67.8 (–C–O–), 62.3 (–C–O–), 26.1 ($-CH_2-$), and 14.1 ($-CH_3$).

(Z)- and (E)-2-(4'-methyl-3'-pentenylidene)-4-butanolide (**3** *and* **4**). Compound **6** (1.86 g, 10 mmol) was dissolved in 30 ml of EtOH at room temperature, and a solution of NaOH (0.40 g, 10 mmol) in 13 ml of water was added. 4-Methyl-3-pentenal (2.94 g, 30 mmol), which had been freshly prepared by acid hydrolysis of 4-methyl-3-pentenal diethyl acetal, was added, and the mixture was stirred at room temperature for 2 days. The solution was diluted with water (90 ml), and 7.0 g of $NaHCO_3$ was added. After being stirred for 30 min at room temperature, the mixture was extracted with ether. The extract was dried over $MgSO_4$ and concentrated to give 1.69 g of a crude oil, which was chromatographed over SiO_2 to yield a 1 : 1 mixture of **3** and **4** (0.30 g, 18%). Further separation by HPLC in an ODS column (eluent of 40% H_2O in MeOH) gave the pure *E*-(**3**, 128.5 mg, 7.7%) and *Z*-(**4**, 119.6 mg, 7.2%) isomers.

E-Isomer (**3**): GLC t_R = 10.44 min; IR ν_{max} (NaCl) cm^{-1}: 2920, 1745, 1365, 1190, 1020; GC/MS *m/z* (%): 41 (100), 53 (44), 67 (31), 79 (72), 91 (78), 105 (42), 123 (68), 151 (27), 166 (M^+, 72); ^1H-NMR (100 MHz) δ_H: 6.69 (1H, tt, 7.6 Hz, 2.9 Hz), 5.13 (1H, t-like, 7.1 Hz), 4.38 (2H, t, 7.3 Hz), 2.89 (4H, 2'-CH_2 overlapped with 3-CH_2, br. t, 7.3 Hz), 1.72 (3H, d, 1.0 Hz), and 1.65 (3H, s); ^{13}C-NMR (25.2 Hz) δ_C: 171.2 (–C=O), 139.2 (–CH=), 134.4 (–C=), 124.7 (–C=), 118.7 (–CH=), 65.2 (–CH_2–O–), 29.1 ($-CH_2-$), 25.5 ($-CH_2-$), 25.0 ($-CH_3$), and 17.7 ($-CH_3$).

Z-Isomer (**4**): GLC t_R = 7.85 min; IR ν_{max} (NaCl) cm^{-1}: 2920, 1740, 1360, 1150, 1080, 1020; GC/MS *m/z* (%): 41 (100), 53 (52), 67 (38), 79 (91), 91 (82), 105 (70), 123 (79), 151 (33), 166 (M^+, 75); ^1H-NMR (100 MHz) δ_H: 6.16 (1H, tt, 7.6 Hz, 2.2 Hz), 5.13 (1H, t-like, 7.3 Hz), 4.32 (2H, t, 7.6 Hz), 3.42 (2H, br. t, 7.6 Hz), 2.92 (2H, ttd, 7.3 Hz, 2.2 Hz, 2.0 Hz), 1.71 (3H, s), and 1.66 (3H, s); ^{13}C-NMR (25.2 Hz) δ_C: 170.0 (–C=O), 141.9 (–CH=), 133.8 (–C=), 122.6 (–C=), 120.2 (–CH=), 65.1 (–CH_2–O–), 28.9 ($-CH_2-$), 26.5 ($-CH_3$), 25.4 ($-CH_2-$), and 17.6 ($-CH_3$).

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References

1) Y. Kuwahara, in "Modern Acarology," Vol. 1, ed. by F. Dasbabek and V. Bukva, Academia, Prague and SPB Academic Pub., The Hague, 1991, pp. 43–52; Y. Kuwahara, *Reports of Chemical Materials Research & Development Foundation*, **10**, 45–52 (1995).
2) W. S. Leal, Y. Kuwahara, T. Suzuki, Y. Nakano, and H. Nakao, *Agric. Biol. Chem.*, **53**, 295–298 (1989); W. S. Leal, Y. Kuwahara, Y. Nakano, H. Nakao, and T. Suzuki, *Agric. Biol. Chem.*, **53**, 1193–1196 (1989); W. S. Leal, Y. Kuwahara, and T. Suzuki, *Naturwissenschaften*, **77**, 387–388 (1990); N. Mori and Y. Kuwahara, *Tetrahedron Letters*, **36**, 1477–1478 (1995); T. Sakata, Y. Kuwahara, and K. Kurosa, *Naturwissenschaften*, **83**, 427 (1996).
3) W. S. Leal, Y. Kuwahara, and T. Suzuki, *Agric. Biol. Chem.*, **53**, 875–878 (1989).
4) W. S. Leal and Y. Kuwahara, in "Modern Acarology," Vol. 2, ed. by F. Dasbabek and V. Bukva, Academia, Prague and SPB Academic Pub., The Hague, 1991, pp. 419–423; M. Sato, Y. Kuwahara, S. Matsuyama, and T. Suzuki, *Biosci. Biotech. Biochem.*, **57**, 1299–1301 (1993).
5) W. S. Leal, Y. Kuwahara, and T. Suzuki, *Agric. Biol. Chem.*, **54**, 2593–2597 (1990).
6) Y. Kuwahara, W. S. Leal, T. Suzuki, M. Maeda, and T. Masutani, *Naturwissenschaften*, **76**, 578–579 (1989).
7) Y. Kuwahara, S. Ishii, and H. Fukami, *Experientia*, **31**, 1115–1116 (1975).
8) L. T. My-Yen, K. Matsumoto, Y. Wada, and Y. Kuwahara, *Appl. Entomol. Zool.*, **15**, 474–477 (1980).
9) W. S. Leal, Y. Nakano, Y. Kuwahara, Y. Nakao, and T. Suzuki, *Appl. Entomol. Zool.*, **23**, 422–427 (1988).
10) Y. Kuwahara, W. S. Leal, A. Akimoto, Y. Nakano, and T. Suzuki, *Appl. Entomol. Zool.*, **23**, 338–344 (1988).
11) R. W. Howard, Y. Kuwahara, H. Suzuki, and T. Suzuki, *Appl. Entomol. Zool.*, **23**, 58–66 (1980).
12) M. Fetizon and M. Golfier, *Compt. Rend.*, **267**, C, 900 (1968); M. Fetizon, M. Golfier, and J.-M, Louis, *J. Chem. Soc., Chem. Commun.*, **1969**, 1118–1119.
13) G. M. Ksander, J. E. McMurry, and M. Johnson, *J. Org. Chem.*, **42**, 1180–1185 (1977).
14) Y. Kuwahara, W. S. Leal, and T. Suzuki, *Jpn. J. Sanit. Zool.*, **41**, 23–28 (1990).
15) S. Noguchi, N. Mori, K. Kurosa, and Y. Kuwahara, *Appl. Entomol. Zool.* (1997), in press.