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STRUCTURE–ACTIVITY RELATIONSHIP OBSERVATIONS FOR THE BAGWORM MOTH PHEROMONE

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Abstract—Structure-activity relationship (SAR) observations were made for the bagworm moth pheromone, (*R*)-2-pentyl decanoate, and a series of analogs with modifications in the alcohol portion of the molecule. Observed attractiveness of these analogs was related to molecular structure and their physical attributes using computational chemistry. Electrostatic potential and Van der Waals (VdW) electrostatic coded surface three-dimensional (3D) maps of the molecular mechanics (MM) minimized lowest energy conformation of the pheromone show that size, shape, charge distribution, and chirality of the molecule are related to attractiveness.

Key Words—SAR, Psychidae, *Thyridopteryx ephemeraeformis*, bagworm moth, sex pheromone, (*R*)-2-pentyl decanoate, Chem-X, molecular modeling.

INTRODUCTION

The bagworm, *Thyridopteryx ephemeraeformis* (Haworth), defoliates ornamentals and trees throughout the Southeast and lower Midwest of the United States (Jones and Parks, 1928). The adult male moth, which flies during the day, is attracted to the adult wormlike female in her pupal case within a silk bag (Neal, 1982) via the female's sex pheromone on her thoracic hairs, which she places at the bottom of the bag until she mates (Leonhardt et al., 1983). After insemination, the female oviposits; the eggs overwinter, and larvae emerge to feed in the spring.

The sex pheromone of the female bagworm moth is (*R*)-2-pentyl decanoate

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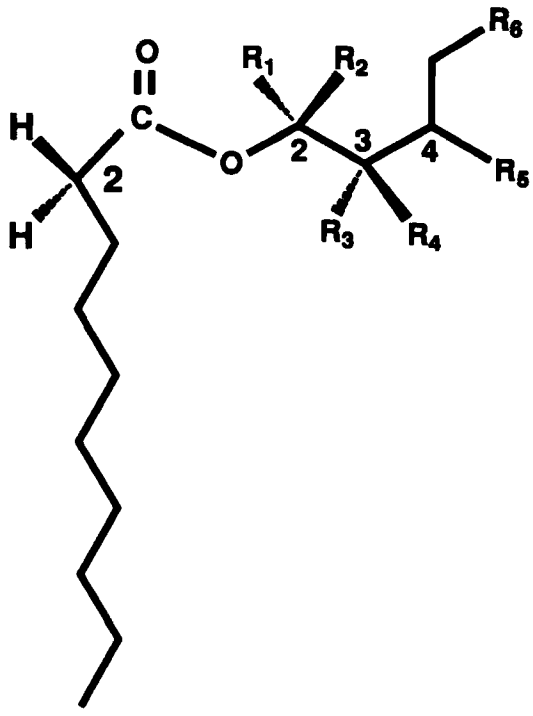

FIG. 1. Bagworm moth pheromone and analogs: (**1a**) $R_1{=}CH_3$, $R_2{=}R_3{=}R_4{=}R_5{=}R_6{=}H$ (pheromone), (**1b**) $R_2{=}CH_3$, $R_1{=}R_3{=}R_4{=}R_5{=}R_6{=}H$ (pheromone antipode), (**2a**) $R_1{=}R_6{=}CH_3$, $R_2{=}R_3{=}R_4{=}R_5{=}H$, (**2b**) $R_2{=}R_6{=}CH_3$, $R_1{=}R_3{=}R_4{=}R_5{=}H$, (**3**) $R_1{=}R_2{=}CH_3$, $R_3{=}R_4{=}R_5{=}R_6{=}H$, (**4a**) $R_1{=}R_3{=}CH_3$, $R_2{=}R_4{=}R_5{=}R_6{=}H$, (**4b**) $R_2{=}R_4{=}CH_3$, $R_1{=}R_3{=}R_5{=}R_6{=}H$, (**4c**) $R_1{=}R_4{=}CH_3$, $R_2{=}R_3{=}R_5{=}R_6{=}H$, (**4d**) $R_2{=}R_3{=}CH_3$, $R_1{=}R_4{=}R_5{=}R_6{=}H$, (**5a**) $R_1{=}R_5{=}CH_3$, $R_2{=}R_3{=}R_4{=}R_6{=}H$, (**5b**) $R_2{=}R_5{=}CH_3$, $R_1{=}R_3{=}R_4{=}R_6{=}H$, (**6**) $R_1{=}R_2{=}R_5{=}CH_3$, $R_3{=}R_4{=}R_6{=}H$, (**7a**) $R_1{=}R_2{=}R_3{=}R_5{=}CH_3$, $R_4{=}R_6{=}H$, and (**7b**) $R_1{=}R_2{=}R_4{=}R_5{=}CH_3$, $R_3{=}R_6{=}H$.

(**1a**) (Figure 1); the antipode (**1b**) is biologically inactive and only acts as a diluent in a racemic mixture (Leonhardt et al., 1983; Klun et al., 1986). The same *R* enantiomeric compound was identified as a sex pheromone in the bagworm, *Oiketicus kirbyi* (Guilding); however, in *O. kirbyi*, the *S* enantiomer inhibited the response of the *R* enantiomer (Rhainds et al., 1994).

To gain information about the structure–activity relationship (SAR) of this chiral pheromone, we report research on the pheromone and a series of analogs based on field studies and computer molecular modeling.

METHODS AND MATERIALS

The bagworm moth pheromone and its analogs (Figure 1) were synthesized via classical esterification procedures (Schwarz and Klun, 1986) with decanoic acid and the following alcohols (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin): (*R*)-2-pentanol, (*S*)-2-pentanol, (±)-2-hexanol, (*R*)-2-hexanol, (*S*)-2-hexanol; 2-methyl-2-pentanol, (±)-3-methyl-2-pentanol, and (±)-4-methyl-2-pentanol. 2,4-Dimethyl-2-pentanol (Wiley Organics, Inc., Coshocton, Ohio) was also used.

(±)-2,3,4-Trimethyl-2-pentanol was synthesized beginning with 3-methylbutanoic acid (isovaleric acid, Aldrich), which was converted to 2,3-dimethylbutanoic acid via butyl lithium in tetrahydrofuran and diisopropylamine to obtain the lithium derivative. The lithium derivative was then treated with methyl iodide and hexamethylphosphoramide (Pfeffer and Silbert, 1970), for an 81.9% yield after distillation at 100°C and 25 torr. The acid chloride formed (Vogel, 1978, p. 498) via thionyl chloride was then converted to the methyl ester with methanol for a 57.8% yield after distillation at 125°C and 760 torr. The desired product was obtained from the methyl ester utilizing methyl magnesium iodide (Vogel, 1978, p. 371).

To determine the purity of the samples, GLC analyses of the analogs (Table 1) were performed on a Shimadzu GC-14A gas chromatograph (GC) equipped with a capillary injector system, a flame ionization detector, and a CR501 data

TABLE 1. PURITY, GLC RETENTION TIME, RETENTION INDEX, AND GC-MS OF RELATED ANALOGS OF FEMALE BAGWORM SEX PHEROMONE

Compound (No.)	Purity (%)[a]	GLC R_t[b]	I_x[c]	e/m[d]
(*R*)-2-Hexyl decanoate (**2a**)	82.6	27.13	1692	M^+-15, 241
(*S*)-2-Hexyl decanoate (**2b**)	92.1	27.15	1693	M^+, 256
(±)-2-Hexyl decanoate (**2a, 2b**)	95.5	27.09	1691	M^+, 256
2-(2-Methyl)pentyl decanoate (**3**)	93.7	25.87	1639	M^+-15, 241
(±)-2-(3-Methyl)pentyl decanoate (**4a–4d**)	46.2	26.70[e]	1674	M^+, 256;
	45.9	26.83[e]	1680	M^+-15, 241
(±)-2-(4-Methyl)pentyl decanoate (**5a, 5b**)	96.9	26.01	1645	M^+, 256
2-(2,4-Dimethyl)pentyl decanoate (**6**)	92.6	27.15	1693	M^+-15, 255
(±)-2-(2,3,4-Trimethyl)pentyl decanoate (**7a, 7b**)	72.2	29.77	1815	M^+, 284

[a] DMS/GLC area that refers to chemical rather than enantiomeric purity.
[b] Dimethylsilicone (DMS), 12 m × 0.2 mm ID fused silica capillary column.
[c] Retention index (Kovats, 1966) on DMS.
[d] DB-1, 60 m × 0.25 mm ID capillary column.
[e] Diastereoisomer.
[f] Diastereoisomers not separable by DB-1.

processor. A Hewlett-Packard 12-m × 0.20-mm-ID dimethylsilicone (DMS) fused silica capillary column was used for the analyses with the following conditions: injector port 200°C; detector 225°C; DMS column, 30°C for 2 min, then programmed at 5°C/min to 180°C and held for 5 min. Total run time was 37 min. A split ratio of 1:50 was used with a helium flow of 1 cm^3/min plus 33 cm^3/min makeup helium. Esters were dissolved in *n*-hexane to make 0.1% solutions, and 1 μl of the solutions was injected for GLC analyses. GC-MS analyses of the analogs (Table 1) were performed on a Finnigan MAT INCOS 50 GC-MS equipped with a Varian 3400 gas chromatograph. The GC component was equipped with a 60-m × 0.25-mm-ID DB-1 capillary column (J&W Scientific) for analyses of the analogs of the female bagworm sex pheromone. The column was held at 100°C for 1 min, then programmed at 20°C/min to 280°C. Analogs were dissolved in *n*-hexane to make 0.01% solutions, and 1 μl of the solutions was injected for GC-MS analyses.

The bagworm female sex pheromone and its analogs were tested for attractiveness for adult males in the field (Table 2). The tests were conducted using Pherocon 1C sticky traps (Trécé, Salinas, California). All traps were baited with rubber stoppers (Thomas Scientific, Catalog no. 1780-J07, Swedesboro, New Jersey) that had been treated with a 60–100 μg/μl methylene chloride solution of compound. In test 1 (Table 2), stoppers were each treated with 1 mg of

TABLE 2. MALE BAGWORM CAPTURES WITH SEX PHEROMONE AND RELATED ANALOGS

Compound (No.)	Total male bagworm captures	
	Test 1, 9/12-10/3/84[a]	Test 2, 10/6-10/21/84[b]
(*R*)-2-Pentyl decanoate[c] (**1a**)	122	245
(*S*)-2-Pentyl decanoate (**1b**)		
(*R*)-2-Hexyl decanoate (**2a**)	43	41
(*S*)-2-Hexyl decanoate (**2b**)	0	0
(±)-2-Hexyl decanoate (**2a, 2b**)	25	22
2-(2-Methyl)pentyl decanoate (**3**)	1	
(±)-2-(3-Methyl)pentyl decanoate (**4a–4d**)	0	
(±)-2-(4-Methyl)pentyl decanoate (**5a, 5b**)	0	
2-(2,4-Dimethyl)pentyl decanoate (**6**)	0	
(±)-2-(2,3,4-Trimethyl)pentyl decanoate (**7a, 7b**)	0	
Blank (empty trap)	1	3

[a] 1 mg/stopper.
[b] 5 mg/stopper.
[c] Female bagworm sex pheromone.

compound; in test 2 (Table 2), stoppers were each treated with 5 mg of compound. Each treated stopper was hung on a paper clip at the center of the trap above its sticky surface. Traps were suspended 1.5 m from the ground and 30 m apart on a chain link fence that surrounded the Beltsville Agricultural Research Center. A randomized complete block design with three replicates and replication over time was used in each test. A blank trap served as the control in both tests. Male captures in the traps were recorded daily or every two days. Freshly treated stoppers were placed in the traps after three to five days in test 1; in test 2, stoppers charged with 5 mg compound were left in the traps throughout the 15 days of the test.

The female bagworm moth pheromone and its analogs were constructed via the computer molecular modeling program, Chem-X (January 1993 VAX version, Chemical Design Ltd, Oxford, England). Lowest-energy conformations were determined using molecular mechanics (Burkert and Allinger, 1982; Clark, 1985) with modified force-field parameters (including Gasteiger charges). Electrostatic potential (Figure 2) and Van der Waals (VdW) electrostatic coded surface three-dimensional (3D) (Figure 3) maps of the bagworm moth pheromone (**1a**) were also constructed.

RESULTS AND DISCUSSION

Previous field tests showed that the *S* enantiomer (**1b**) of the levorotatory bagworm moth pheromone, (*R*)-2-pentyl decanoate (**1a**) (Figure 1, Table 1), is inactive and does not inhibit the attractiveness of the *R* enantiomer (Leonhardt et al., 1983; Klun et al., 1986).

To study the SAR of the pheromone (**1a**), modifications were made in the alcohol portion of the molecule. The alcohol chain was extended by one carbon via synthesis of (±)-1-hexyl decanoate (**2a, 2b**). This racemate was ~20% as active (test 1, Table 2) as the pheromone; the levorotatory *R* (**2a**) enantiomer of this analog was ~35% as active as the pheromone, and the *S* (**2b**) enantiomer was inactive. To confirm the latter findings, test 2 was conducted; the levorotatory *R* enantiomer (**2a**) was 17% as active as the pheromone, and the *S* (**2b**) enantiomer was inactive. In both tests, the levorotatory *R* enatiomer (**2a**) was about twice as active as the racemate (**2a, 2b**). This indicated that the inactive *S* enantiomer was not inhibitory to the *R* enantiomer (**2a**).

Other structural changes to the pheromone molecule were also considered, which contributed to the SAR study. A carbon was added to the pheromone at the 2-pentyl position to give 2-(2-methyl)pentyl decanoate (**3**), which was essentially inactive in field traps. A carbon was added to the pheromone at the 3-pentyl position to give (±)-2-(3-methyl)pentyl decanoate (**4a–4d**), which was inactive. Similarly, a carbon was added to the pheromone at the 4-pentyl position

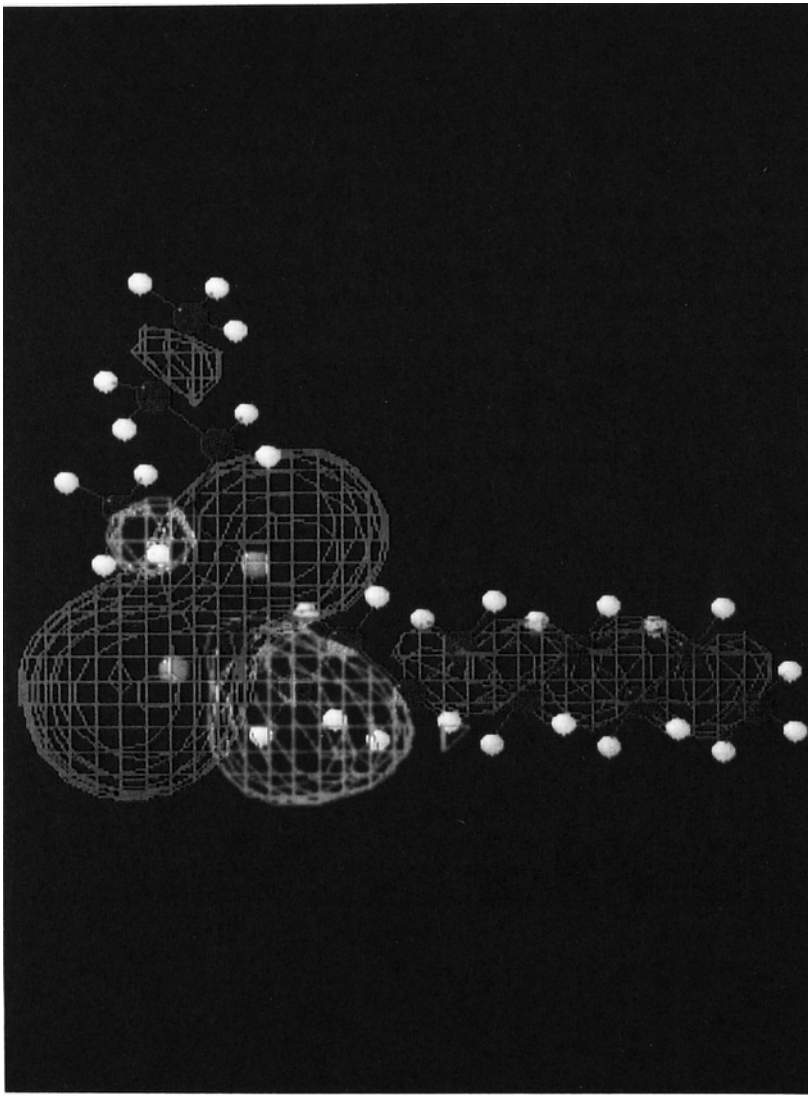

FIG. 2. Electrostatic potential 3D map of bagworm moth pheromone (**1a**). Standard contours: cyano, < -10.0 kcal; red, > 10.0 kcal; significance = 20; grid density = 2.5 contours/Å.

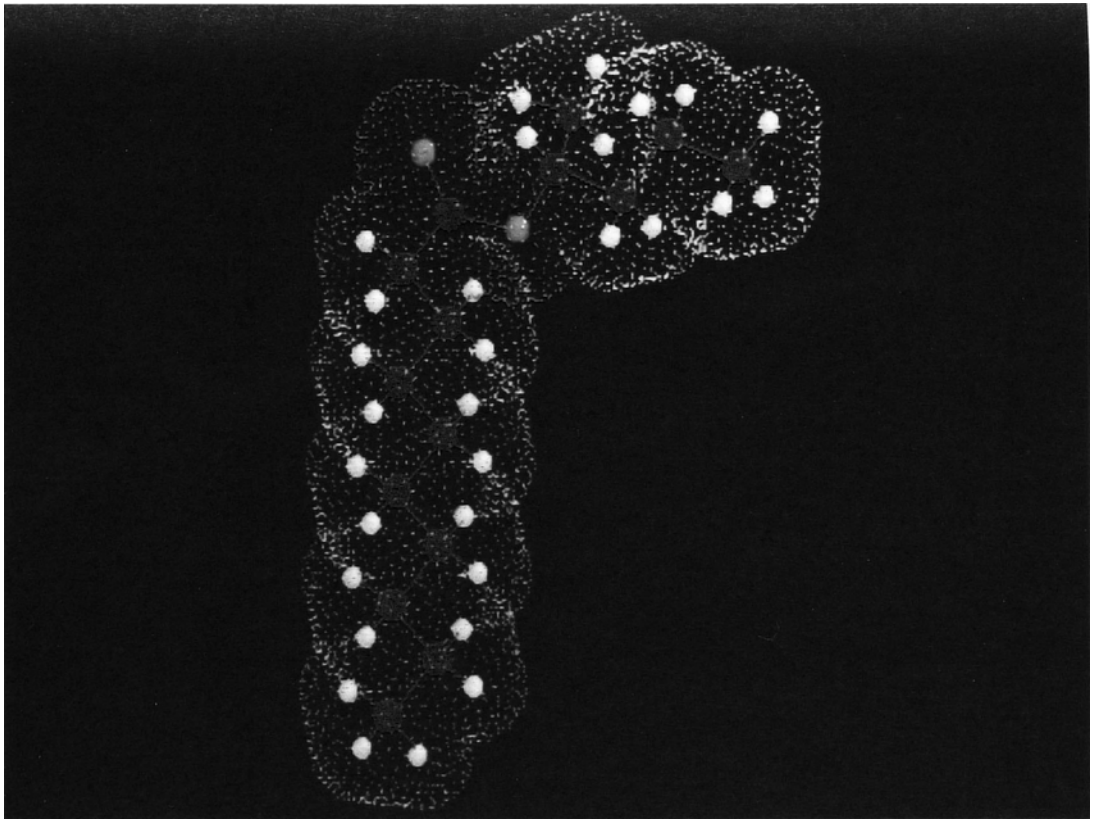

FIG. 3. VdW electrostatic coded surface 3D map of bagworm moth pheromone (**1a**). Standard contours: cyano, < -20.0 kcal; green, < -10.0 kcal; white, < 10.0 kcal; yellow, < 20.0 kcal; red, > 20.0 kcal; significance = 0.95; grid density = 2.5 contours/Å.

to give (±)-2-(4-methyl)pentyl decanoate (**5a, 5b**), which was also inactive; two carbons were added to the basic pheromone structure to give 2-(2,4-dimethy)pentyl decanoate (**6**), which was inactive. Finally, three carbons were added to the basic pheromone structure to give (±)-2-(2,3,4-trimethyl)pentyl decanoate (**7a, 7b**) which was also inactive.

The GLC purity (Table 1) of the analogs was 92+% except for (*R*)-2-hexyl decanoate (**2a**) and (±)-2-(2,3,4-trimethyl)pentyl decanoate (**7a, 7b**), which were 82.6 and 72.2%, respectively. The two diastereoisomers of (±)-2-(3-methyl)pentyl decanoate (**4a–d**) were resolved on the 12-m × 0.20-mm-ID column, but not on the 60-m × 0.25-mm-ID column with the conditions used. All analogs had a parent ion in the GC-MS, except for **2a, 3,** and **6,** which had $M^+ - 15$ ions.

The electrostatic potential map (Figure 2) of the pheromone (MM energy = −1.2071 kcal/mol), (*R*)-2-pentyl decanoate (**1a**), shows partially charged positive areas on the hydrogen at the (*R*)-2 position and on the hydrogens at the α-position of the decanoate chain. There is also a partially charged negative area around the two O atoms because of the electronegativity of these atoms. This could indicate that not only is the chiral steric effect of the (*R*)-2-CH_3 necessary for attractancy, but so is the partially charged positive (*R*)-2-H in relation to the partially charged negative oxygens with adjacent partially charged positive α-hydrogens on the decanoate chain. The volumes of the cyano (< −10 kcal electrostatic potential) and red contours (> 10 kcal electrostatic potential) are 67.8989 $Å^3$ and 17.9873 $Å^3$, respectively; the surface areas of the cyano and red contours are 97.4782 $Å^2$ and 30.7097 $Å^2$, respectively.

The VdW electrostatic coded surface 3D map (Figure 3) of the pheromone gives an indication of the size (volume = 216.47 $Å^3$), surface shape (area = 230.312 $Å^2$), and electrostatic charge distribution that is required for biological activity.

The antipode (**1b**) of the pheromone and **2b** are inactive because they appear right-handed instead of left-handed, with electrostatic potentials on the (*S*)-2-H and the α-hydrogens of the decanoate that are reversed in relation to the potential on the oxygens. In **1a** and **1b,** the energy minimized structure is L-shaped (Figure 3). Chirality determines whether the front or back surface of the L shape contains the electropositive 1-H proton. The large difference in their activity suggests only one surface (i.e., front) is active. Compound **2a** has the correct chirality for activity, but is tempered in activity by steric effects corresponding to extra molecular volume and surface area of a terminal methyl. Analogs that are achiral at position-2 (**3, 6,** and **7a, 7b**) lack the (*R*)-2-H to give the appropriate electrostatic potential distribution. The remaining compounds (**4a–d** and **5a, 5b**) have an (*R*)-2-H in the racemate, but the added substituents cause an increase in the molecular volume and surface area that could exceed the requirements. The volatilities of compounds (Tables 1 and 2)

would be expected to be quite similar except for **7a** and **7b**, with a retention time of 2.6–3.9 min greater than the other compounds.

No modifications were attempted on the acid portion of the pheromone for this study.

CONCLUSIONS

Modifications in the alcohol portion of the levorotatory bagworm moth pheromone, (*R*)-2-pentyl decanoate (**1a**), revealed that the chirality at the 2-pentyl position is absolutely essential for attractiveness since the *S* antipode (**1b**) is not attractive. It was also determined that the most stable conformation is L-shaped. Lengthening the alcohol with a terminal carbon reduced attractiveness by 65–83% as long as the correct chirality (**2a**) was maintained. Steric effects from the addition of a carbon at the 2 (**3**), 3 (**4a–d**), or 4 positions (**5a, 5b**); two carbons at the 2 and 4 positions (**6**); or three carbons at the 2, 3, and 4 positions (**7a, 7b**) of the pheromone resulted in no attractiveness. The bagworm moth allows very few changes in the alcohol portion of the pheromone if attractancy is to be maintained. The dumbbell shape of the electrostatic partial negative charge of the oxygen atoms in relation to the electrostatic partial positive charge of the chiral (*R*)-2-H and the electrostatic partial positive charge of the α-hydrogens on the decanoate chain point toward the importance of the front surface of the L-shaped (**1a**) enantiomer. Size and shape linked to this chiral surface contribute to its attractiveness for bagworm moths.

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