# First Total Synthesis of Artepillin C Established by *o,o′*-Diprenylation of *p*-Halophenols in Water

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**Abstract:** We have demonstrated that prenylation of *p*-halophenols was dependent on the solvent effect and succeeded in *o,o′*-diprenylation of *p*-halophenols in water. Following the Mizoroki−Heck coupling of the diprenyl-*p*-iodophenol **3c** with methyl acrylate and then hydrolysis, we first synthesized artepillin C [3-{4-hydroxy-3,5-di(3-methyl-2-butenyl)phenyl}-2(*E*)-propenoic acid] (**1**), which is a biologically active constituent of propolis. These reactions may be applicable to the synthesis of various useful natural products such as 2,4,6-trisubstituted phenol derivatives.

Propolis, obtained from beehives, consists of a mixture of compounds that includes various 2,4,6-trisubstituted phenols. Because of its well-known biological effects such as antibacterial, antiviral, antiinflammatory, antioxidative, and immunomodulatory effects, propolis has been used as a popular medicine. Artepillin C [3-{4-hydroxy-3,5-di(3-methyl-2-butenyl)phenyl}-2(*E*)-propenoic acid] (**1**) is an antimicrobial 2,4,6-trisubstituted phenol isolated as a major constituent (>5%) from Brazilian propolis.[1] The recent report that artepillin C has important biological activities such as antitumor,[2] apoptosis-inducing,[3] immunomodulating,[4] and antioxidative activities[5] shows that artepillin C may be one of the important active principles of Brazilian propolis. Indeed, the importance of artepillin C has been increasingly recognized and it has recently been designated a criterion of the quality of Brazilian propolis. These biological properties have made clear the urgent need for an efficient total synthesis of artepillin C. However, to the best of our knowledge, the total synthesis of artepillin C has never been accomplished. Our attempts to achieve a direct synthesis of artepillin C by *o,o′*-diprenylation of *p*-hydroxycinnamic acid were thwarted in part because of difficulty in isolation of pure artepillin C from the reaction mixture. This was due to the large amount of prenyl bromide and byproducts in the reaction mixture. Moreover, the α,*β*-unsaturated carboxyl group of *p*-hydroxycinnamic acid is not an ideal functional group for the further modifications required for our synthesis.

We here present the first total synthesis of artepillin C. Our synthetic strategy was based on an initial *o,o′*-diprenylation reaction of *p*-halophenols. The advantage of this approach is that fewer side reactions occur in the reaction of *p*-halophenols than *p*-hydroxycinnamic acid, and that product isolation and purification are greatly simplified. Our short and efficient synthesis of artepillin C was completed by Mizoroki−Heck reaction of the isolated 2,6-di(3-methyl-2-butenyl)-4-halophenols (*o,o′*-diprenyl-*p*-halophenols) with methyl acrylate followed by hydrolysis of the ester.

Concerning the *o*-prenylation of phenol derivatives, Fatope et al. reported that prenylation of 4-(*p*-hydroxyphenyl)butyric acid in organic solvents such as tetrahydrofuran or toluene gave 3-prenyl-4-(*p*-hydroxyphenyl)-butyric acid and no *o,o′*-diprenyl compound was obtained.[6] Bates et al. also reported that prenylation of *p*-halophenols in toluene gave *o*-prenyl-*p*-halophenols, and no *o,o′*-diprenyl compounds were obtained.[7] Lewis et al. achieved initial success of the *o,o′*-dialkylation of phenol with ethylene. However, their procedure, use of an *o*-metalated ruthenium phosphite complex under a pressure of 6.5 bar, is not a convenient laboratory operation.[8]

In the work we present here, we reveal that *o,o′*-diprenylation of *p*-halophenols can be readily achieved at room temperature in alkaline solution (Scheme 1). First, we focused on the effect of the nature of the halogen on reactivity and regioselectivity of *C*- and *O*-prenylation of *p*-halophenols. The results are summarized in Table 1. The yield of *o,o′*-diprenyl-*p*-iodophenol **3c** (24%, entry 3) was the highest, followed by that of chlorophenol **3a** (16%, entry 1) and bromophenol **3b** (15%, entry 2). Ratios of *C*- to *O*-prenylation of **2a**, **2b**, and **2c** were 1.3, 1.5, and 1.6, respectively. Next, we tried to obtain the *o,o′*-diprenyl-*p*-halophenols selectively with **2c** as a starting material. When **2c** was treated with 1.0 equiv of prenyl bromide for 1 h, **3c** was obtained selectively (27%) and byproducts **4c** and **5c** were obtained in very low yield (2 and 3%, respectively), while byproduct **6c** (7%) was newly obtained (entry 4). The conversion yield of **2c** to **3c** was 47% (**2c** was recovered in 42%).

To understand the differences in reactivity between *p*-halophenols and *p*-hydroxycinnamic acid in the *o,o′*-diprenylation reaction, we calculated their molecular orbital energies using the AM1 method on the MOPAC97 program (Figure 1). The highest occupied molecular orbital (HOMO) energy of **2a′**−**c′** (from −3.040 to −3.246 eV) was close to the lowest unoccupied molecular orbital (LUMO) energy of prenyl bromide (0.841 eV). Moreover, **2a′**−**c′** showed high $C^2$ values at their ortho (from 0.213 to 0.216) and para positions (from 0.307 to 0.319). Thus, *p*-halophenols suffered electrophilic attack by prenyl bromide at their ortho positions. However, the order of reactivity of *o,o′*-diprenylation was opposite that predicted by the nucleophilicities of ortho positions obtained

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**Table 1. Product Distributions Obtained in the Prenylation of *p*-Halophenols 2a−c**

| | | | | products[a] (isolated yield/%) | | | | |
|---|---|---|---|---|---|---|---|---|
| entry | **2** | prenyl bromide (equiv) | time (h) | **3** | **4** | **5** | **6** | recovered **2** |
| 1 | **2a** | 2.5 | 5 | **3a** (16, 23[b]) | **4a** (20) | **5a** (35) | | 32 |
| 2 | **2b** | 2.5 | 5 | **3b** (15, 23[b]) | **4b** (16) | **5b** (26) | | 36 |
| 3 | **2c** | 2.5 | 5 | **3c** (24, 29[b]) | **4c** (23) | **5c** (35) | | 17 |
| 4 | **2c** | 1.0 | 1 | **3c** (27, 47[b]) | **4c** (2) | **5c** (3) | **6c** (7) | 42 |

[a] Assignment of structures is based on NMR data (see Experimental Section). [b] Conversion yield of **2** to **3**.

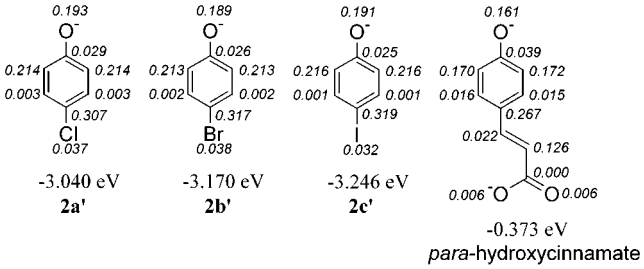
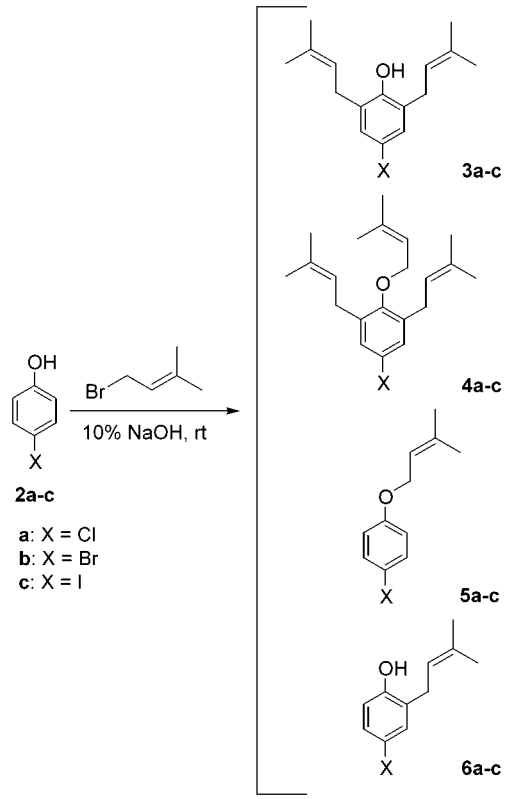



**Figure 1.** HOMO energies and the square of atomic orbital coefficients ($C^2$) calculated for phenolates **2a′**−**c′** and *p*-hydroxycinnamate.

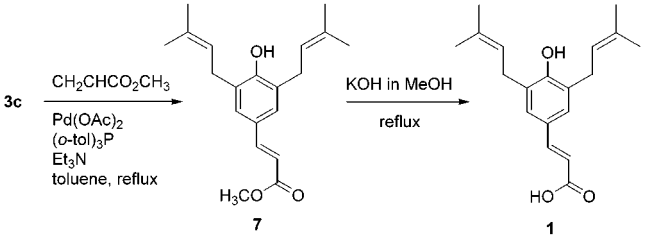
**Scheme 1. Prenylation of *p*-Halophenols 2a−c**




from calculated HOMO energies. This finding suggested that the halogen group was influenced by the protic solvent. However, our calculation did not consider the solvent effect. In the regioselectivity of *C*- or *O*-prenylation, a difference in the $C^2$ values of hydroxyl anion (0.193) and ortho positions (0.214) of **2a′** agreed closely with the observed *C*- to *O*-prenylation ratio.

Additionally, the HOMO energy of *p*-hydroxycinnamate (−0.373 eV) is higher than those of phenolates **2a′**−**c′**. This finding predicted that *p*-hydroxycinnamic acid actually would have a higher reactivity toward prenyl bromide than *p*-halophenols. However, as discussed above, *o*,*o′*-diprenylated *p*-hydroxycinnamic acid was obtained in low yield.

**Scheme 2. Synthesis of Artepillin C 1 by Mizoroki−Heck Reaction**




Next, we examined the Mizoroki−Heck coupling of *o*,*o′*-diprenyl-*p*-halophenols **3a**−**c** with methyl acrylate. Mizoroki−Heck coupling of aromatic rings bearing an electron-donating substituent such as the hydroxyl group with an alkene is generally inefficient because of slow alkene coupling and decomposition of the palladium catalyst.[9] Therefore, we first acetylated the hydroxyl group of **3a**−**c** to reduce the electron density of the ring, but we did not obtain any coupling products. This result may be due to the steric hindrance of two *o*-prenyl groups against the hydroxyl group.

We finally were able to effect Mizoroki−Heck coupling of *o*,*o′*-diprenyl-*p*-halophenols **3c** with methyl acrylate without protection of its hydroxyl group by using 5 mol % palladium diacetate, 10 mol % tri-*o*-tolylphosphine, and an excess of triethylamine to produce artepillin C methyl ester **7** in 31% yield (Scheme 2). In the case of **3a** and **3b**, only starting materials were recovered under similar conditions. Our results are consistent with those of Bates et al., who reported that, without protection of the hydroxyl group, the Mizoroki−Heck coupling proceeded only with *p*-iodophenol derivatives.[7] Finally, artepillin C methyl ester **7** was hydrolyzed in aqueous/methanolic potassium hydroxide to give artepillin C **1** in 78% yield.

In summary, the total synthesis of artepillin C was achieved by *o*,*o′*-diprenylation of *p*-iodophenol in water followed by Mizoroki−Heck coupling with methyl acrylate. In future work, we will apply this strategy to the synthesis of other biologically active 2,4,6-trisubsutituted phenol derivatives.

## Experimental Section

All commercial materials were used without purification. Toluene was distilled under nitrogen from $CaH_2$. Analytical thin-layer chromatography was performed using silica 60-$F_{254}$ coated 0.25 mm plates. Column chromatography was performed using the indicated solvent on silica gel (63−200 $\mu$m). $^1$H NMR (400 Mz) spectra were recorded using diluted solutions of each compound in $CDCl_3$ as the solvent and tetramethylsilane as the internal standard. All melting points were determined by a micro melting point apparatus and are uncorrected. The semiempirical molecular orbital (MO) calculation was applied by the AM1

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methods of Stewart and the program MOPAC97. The orbital energy (eV) and $C^2$ value of the highest occupied molecular orbital (HOMO) were calculated. The *o*-prenyl-*p*-iodophenol **6c** is a known compound.[7]

**Prenylation of 4-Halophenols 2a−c. General Procedure.** The corresponding *p*-substituted phenol (1.0 mmol) was dissolved in 10% NaOH (2.4 mL per mol of *p*-substituted phenol), and prenyl bromide (2.5 or 1.0 mol per mol of *p*-substituted phenol) was added dropwise. After the reaction mixture was stirred at room temperature for the indicated period of time, the mixture was cooled to 0−5 °C in an ice-bath and then acidified with 2 M $CH_3COOH$. The mixture was extracted with $Et_2O$ and washed with $H_2O$. The $Et_2O$ layer was dried ($MgSO_4$) and evaporated under reduced pressure. Residues were purified by column chromatography on silica gel (hexanes/$Et_2O$, 20:1). After preliminary examination of the eluates by TLC, the following major products (in order of elution) were obtained.

**4-Chloro-2,6-di(3-methyl-2-butenyl)phenyl (3-Methyl-2-butenyl) Ether (4a):** colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.97 (s, 2H, $H_3$,$H_5$−Ar), 5.56 (t, $J$ = 7.1 Hz, 1H, $OCH_2CH$=$C(CH_3)_2$), 5.25 (t, $J$ = 7.3 Hz, 2H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 4.27 (d, $J$ = 7.1 Hz, 2H, $OCH_2CH$=$C(CH_3)_2$), 3.34 (d, $J$ = 7.3 Hz, 4H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 1.79 (s, 3H, $CH_3$), 1.75 (s, 6H, $C(CH_3)_2$), 1.71 (s, 6H, $C(CH_3)_2$), 1.68 (s, 3H, $CH_3$); EI-MS $m/z$ 332 ($M^+$). Anal. Calcd for $C_{21}H_{29}ClO$: C, 75.76; H, 8.78. Found: C, 75.96; H, 8.68.

**4-Bromo-2,6-di(3-methyl-2-butenyl)phenyl (3-Methyl-2-butenyl) Ether (4b):** colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.12 (s, 2H, $H_3$,$H_5$−Ar), 5.56 (t, $J$ = 7.1 Hz, 1H, $OCH_2CH$=$C(CH_3)_2$), 5.25 (t, $J$ = 7.1 Hz, 2H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 4.27 (d, $J$ = 7.1 Hz, 2H, $OCH_2CH$=$C(CH_3)_2$), 3.34 (d, $J$ = 7.1 Hz, 4H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 1.79 (s, 3H, $CH_3$), 1.75 (s, 6H, $C(CH_3)_2$), 1.71 (s, 6H, $C(CH_3)_2$), 1.68 (s, 3H, $CH_3$); EI-MS $m/z$ 376 ($M^+$). Anal. Calcd for $C_{21}H_{29}BrO$: C, 66.84; H, 7.75. Found: C, 67.03; H, 7.57.

**4-Iodo-2,6-di(3-methyl-2-butenyl)phenyl (3-Methyl-2-butenyl) Ether (4c):** colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.31 (s, 2H, $H_3$,$H_5$−Ar), 5.55 (t, $J$ = 7.1 Hz, 1H, $OCH_2CH$=$C(CH_3)_2$), 5.24 (t, $J$ = 7.1 Hz, 2H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 4.27 (d, $J$ = 7.1 Hz, 2H, $OCH_2CH$=$C(CH_3)_2$), 3.32 (d, $J$ = 7.1 Hz, 4H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 1.79 (s, 3H, $CH_3$), 1.75 (s, 6H, $C(CH_3)_2$), 1.71 (s, 6H, $C(CH_3)_2$), 1.68 (s, 3H, $CH_3$); EI-MS $m/z$ 424 ($M^+$). Anal. Calcd for $C_{21}H_{29}IO$: C, 59.44; H, 6.89. Found: C, 59.68; H, 6.72.

**1-Chloro-4-[(3-methyl-2-butenyl)oxy]benzene (5a):** colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.23 (d, $J$ = 9.3 Hz, 2H, $H_3$,$H_5$−Ar), 6.84 (d, $J$ = 9.3 Hz, 2H, $H_2$,$H_6$−Ar), 5.49 (t, $J$ = 6.8 Hz, 1H, $OCH_2CH$=$C(CH_3)_2$), 4.49 (d, $J$ = 6.8 Hz, 2H, $OCH_2CH$=$C(CH_3)_2$), 1.81 (s, 3H, $CH_3$), 1.75 (s, 3H, $CH_3$); EI-MS $m/z$ 196 ($M^+$). Anal. Calcd for $C_{11}H_{13}ClO$: C, 67.18; H, 6.66. Found: C, 67.22; H, 6.63.

**1-Bromo-4-[(3-methyl-2-butenyl)oxy]benzene (5b):** colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.36 (d, $J$ = 8.8 Hz, 2H, $H_3$,$H_5$−Ar), 6.79 (d, $J$ = 8.8 Hz, 2H, $H_2$,$H_6$−Ar), 5.46 (t, $J$ = 6.6 Hz, 1H, $OCH_2CH$=$C(CH_3)_2$), 4.47 (d, $J$ = 6.6 Hz, 2H, $OCH_2CH$=$C(CH_3)_2$), 1.79 (s, 3H, $CH_3$), 1.73 (s, 3H, $CH_3$); EI-MS $m/z$ 240 ($M^+$). Anal. Calcd for $C_{11}H_{13}BrO$: C, 54.79; H, 5.43. Found: C, 55.02; H, 5.42.

**1-Iodo-4-[(3-methyl-2-butenyl)oxy]benzene (5c):** colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.54 (d, $J$ = 8.8 Hz, 2H, $H_3$,$H_5$−Ar), 6.69 (d, $J$ = 8.8 Hz, 2H, $H_2$,$H_6$−Ar), 5.46 (t, $J$ = 6.8 Hz, 1H, $OCH_2CH$=$C(CH_3)_2$), 4.47 (d, $J$ = 6.8 Hz, 2H, $OCH_2CH$=$C(CH_3)_2$), 1.79 (s, 3H, $CH_3$), 1.73 (s, 3H, $CH_3$); EI-MS $m/z$ 288 ($M^+$). Anal. Calcd for $C_{11}H_{13}IO$: C, 45.85; H, 4.55. Found: C, 46.05; H, 4.49.

**4-Chloro-2,6-di(3-methyl-2-butenyl)phenol (3a):** yellow oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.94 (s, 2H, $H_3$,$H_5$−Ar), 5.31 (s, 1H, O*H*), 5.27 (t, $J$ = 7.1 Hz, 2H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 3.29 (d, $J$ = 7.1 Hz, 4H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 1.77 (s, 6H, $C(CH_3)_2$), 1.75 (s, 6H, $C(CH_3)_2$); EI-MS $m/z$ 264 ($M^+$). Anal. Calcd for $C_{16}H_{21}ClO$: C, 72.57; H, 7.99. Found: C, 72.31; H, 7.88.

**4-Bromo-2,6-di(3-methyl-2-butenyl)phenol (3b):** yellow oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.08 (s, 2H, $H_3$,$H_5$−Ar), 5.34 (s, 1H, O*H*), 5.27 (t, $J$ = 7.3 Hz, 2H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 3.29 (d, $J$ = 7.3 Hz, 4H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 1.77 (s, 6H, $C(CH_3)_2$), 1.75 (s, 6H, $C(CH_3)_2$); EI-MS $m/z$ 308 ($M^+$). Anal. Calcd for $C_{16}H_{21}BrO$: C, 62.14; H, 6.84. Found: C, 62.35; H, 6.87.

**4-Iodo-2,6-di(3-methyl-2-butenyl)phenol (3c):** yellow oil; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.26 (s, 2H, $H_3$,$H_5$−Ar), 5.36 (s, 1H, O*H*), 5.26 (t, $J$ = 6.8 Hz, 2H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 3.27 (d, $J$ = 6.8 Hz, 4H, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 1.77 (s, 6H, $C(CH_3)_2$), 1.75 (s, 6H, $C(CH_3)_2$); FAB−HRMS (glycerol matrix) $m/z$ calcd for $C_{16}H_{21}IO$ [$(M + H)^+$] 356.0637, found 356.0654. Anal. Calcd for $C_{16}H_{21}IO$: C, 53.94; H, 5.94. Found: C, 53.79; H, 5.69.

**3-{4-Hydroxy-3,5-di(3-methyl-2-butenyl)phenyl}-2(*E*)-propenoic Acid (1).** A solution of **3c** (1.67 g, 4.69 mmol), methyl acrylate (2.10 mL, 23.3 mmol), triethylamine (1.30 mL, 9.33 mmol), tri-*o*-tolylphosphine (143 mg, 0.470 mmol), and palladium diacetate (52 mg, 0.232 mmol) in dry toluene (7 mL) was heated at 100 °C for 20 h. The mixture was cooled to room temperature, diluted with EtOAc/$Et_2O$, and filtered through Celite. The filtrate was washed with saturated $NH_4Cl$ solution. The aqueous layer was reextracted with EtOAc/$Et_2O$. The combined organic layers were dried ($MgSO_4$) and evaporated under reduced pressure. Residues were purified by column chromatography on silica gel (hexanes/EtOAc, 10:1) to give 521 mg (1.46 mmol, 31%) of product **7** as a brown oil. KOH (15 mL of a 10% aqueous solution) was added to a solution of the methyl ester **7** (274 mg, 0.769 mmol) in MeOH (15 mL). The mixture was heated under reflux for 1 h, cooled to 0−5 °C, and acidified with 1 N HCl. The MeOH was evaporated under reduced pressure, and the aqueous residue was extracted with EtOAc. Extracts were washed with saturated $NH_4Cl$ and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 20:1) to give 181 mg (0.60 mmol, 78%) of product **1** as a white solid: mp 98−99 °C; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.69 (d, 1H, $J$ = 15.6 Hz, $ArCH$=), 7.20 (s, 2H, $H_3$,$H_5$−Ar), 6.29 (d, 1H, $J$ = 15.6 Hz, $CHCO_2H$), 5.31 (t, 2H, $J$ = 6.8 Hz, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 3.35 (d, 4H, $J$ = 6.8 Hz, $Ar(CH_2CH$=$C(CH_3)_2)_2$), 1.79 (s, 6H, $C(CH_3)_2$), 1.78 (s, 6H, $C(CH_3)_2$); FAB−HRMS (glycerol matrix) $m/z$ calcd for $C_{19}H_{24}O_3$ [$(M + H)^+$] 300.1725, found 300.1682. Anal. Calcd for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 75.75; H, 7.89.

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