Palythoalones A and B, New Ecdysteroids from the Marine Zoanthid *Palythoa australiae*

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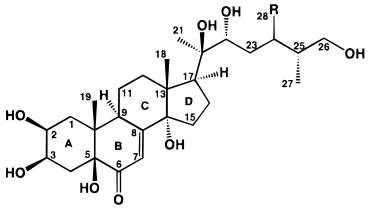
Two new ecdysteroids, palythoalones A (**1**) and B (**2**), have been isolated from the marine zoanthid *Palythoa australiae*. The structures have been elucidated on the basis of spectroscopic data and by chemical means.

Relatively few chemical studies on constituents of marine zoanthids have been reported.[1] In our continuing search for bioactive substances from marine organisms,[2] we have examined extracts of a zoanthid *Palythoa australiae* Carlgren, and have isolated two new ecdysteroids, palythoalones A (**1**) and B (**2**). In this paper, the isolation and structure elucidation of **1** and **2** are described.



1 R = Me
2 R = H

The methanolic extract of the zoanthid *P. australiae* collected off Seragaki, Okinawa, was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was purified by successive chromatographic steps on a Si gel column followed by Si gel HPLC to afford palythoalones A (**1**, 0.014%) and B (**2**, 0.00060%), together with known ecdysteroids, makisterone B[3] and inokosterone,[4] previously isolated from terrestrial plants.

Palythoalone A (**1**) was obtained as a colorless amorphous solid, and the molecular formula was established as $C_{28}H_{46}O_8$ by HRFABMS (*m/z* 511.3263 $[M + H]^+$, Δ −0.8 mmu). IR absorptions implied that **1** possessed hydroxyl (3435 cm^{-1}) and unsaturated carbonyl (1635 cm^{-1}) groups. The presence of an α,*β*-unsaturated carbonyl group was also indicated by the UV spectrum [λ_{max} 243 nm (ϵ 10 000)]. Analysis of the ^1H and ^{13}C NMR data and the HMQC spectrum provided evidence that **1** possessed five methyl groups, one α,*β*-unsaturated ketone, three oxygenated quaternary carbons, three oxymethines, one oxymethylene, seven methylenes, four methines, and two quaternary carbons. The $^1H-^1H$ COSY spectrum revealed the connectivities of C-1 to C-4, C-9 to C-12, C-15 to C-17, C-22 to C-24, and C-25 to C-27. In the HMBC spectrum, long-range $^1H-^{13}C$ correlations of H_3-19 to C-1, C-5, C-9, and C-10 indicated that Me-19 was connected to C-10. HMBC correlations of H-1b and H-3 to C-5 (δ 80.0) revealed that a hydroxyl group was attached at C-5 on a cyclohexane ring (ring A), while the correlations of H-4 and H-7 to C-6 (δ 200.9) and H-7 to C-9 revealed the presence of another cyclohexenone ring (ring B). HMBC cross-peaks of H_3-18 to C-12, C-13, C-14, C-17 and of H-7 and H-15 to C-14 indicated that Me-18 and the hydroxyl group were attached at C-13 and C-14, respectively. $^1H-^{13}C$ correlations of H_3-21 and H-22 to C-20 and H-25 to C-24 revealed the presence of a side-chain (C-20−C-28) with two hydroxyl groups at C-20 (δ 76.8) and C-22 (δ 74.1) and three methyls at C-20, C-24, and C-25. The connectivity between C-17 and C-20 of the side-chain was supported by HMBC correlations of H_3-21 to C-17 and H-17 to C-20. Thus, the structure of palythoalone A (**1**) was assigned as **1**. The relative stereochemistry of **1** was deduced from NOESY data and $^1H-^1H$ coupling constants (Figure 1). Chair conformations for both of rings A and B and a *cis*-junction between them were elucidated from the coupling constant (11.8 Hz) between H-1a and H-2, a broad singlet of H-3, and NOESY correlations of H-2 to H-4a, H_3-19 to H-11a, H-9 to H-12a, and H-12b to H_3-18. The hydroxyl groups at C-2 and C-3 were assigned as being *β*-oriented, judging from the coupling constant between H-2 and H-3. A NOESY correlation between H-12a and H-17 indicated that the C-20−C-28 side-chain was *β*-oriented. Comparison of ^{13}C NMR chemical shifts of C-20 and C-22 (δ_C 76.8, s; 74.1, d, respectively) in **1** with those of the four diastereomers of 20,22-dihydroxy sterols previously reported indicated the presence of 20*β* and 22α-configuration.[5] The absolute configuration of the tetracyclic part in **1** was elucidated from the CD spectrum. The CD curves of **1** showed positive Cotton effects, θ + 9100 at 329 nm and θ −14 000 at 254 nm, for the $n-\pi^*$ transition of the CO group (R-band). The CD spectrum of **1** was similar to those of sengosterone and cyasterone,[6] suggesting that the absolute configurations of the tetracyclic part are as shown in Figure 1. The 2,3- and 20,22-diacetonide derivative (**3**) of **1** was treated with (+)- and (−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chlorides (MTPA chlorides) to afford the 26-*O*-(+)- and 26-*O*-(−)-MTPA esters **4a** and **4b**, respectively (Scheme 1). Comparison of the chemical shift difference (Δ 0.12 ppm) of the methylene proton signals at C-26 of **4a** observed at δ 4.17 and 4.29 with that (Δ 0.03 ppm) of **4b** at δ 4.23 and

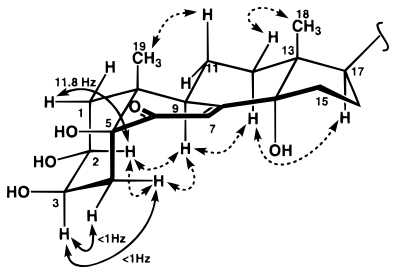


Figure 1. Relative stereochemistry of palythoalone A (**1**) (dotted arrows denote NOESY correlations).

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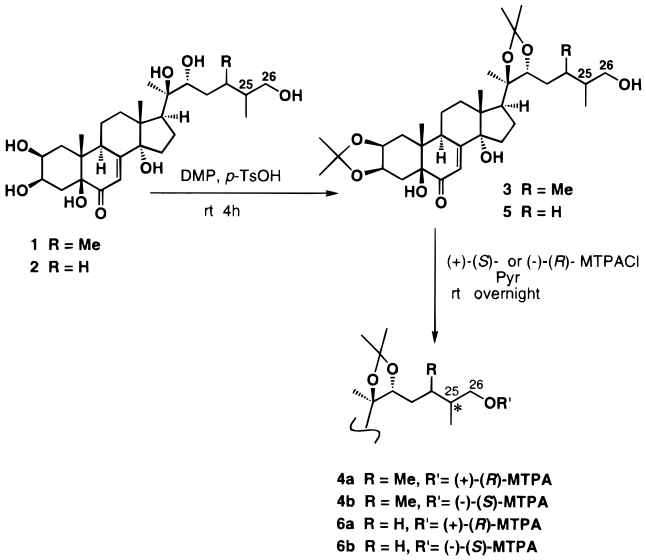
Scheme 1. Preparation of diacetonides (**3** and **5**) of palythoalones A (**1**) and B (**2**) and their MTPA derivatives (**4a** and **4b**; **6a** and **6b**).



4.26 indicated the 25*R* configuration of **1**,[7] while it was difficult to determine the absolute configuration at C-24.

Palythoalone B (**2**) showed a pseudomolecular ion peak at *m*/*z* 497 $[M + H]^+$ in the FABMS, and the molecular formula, $C_{27}H_{44}O_8$, was established by HRFABMS (*m*/*z* 497.3118 $[M + H]^+$, Δ +0.3 mmu), indicating that **2** was the demethyl analogue of **1**. The ^1H, ^{13}C, and 2D NMR data of the tetracyclic part in **2** were similar to those of **1**. $^1H-^1H$ COSY and HMBC correlations of **2** implied connectivities of C-22 to C-27, indicating that **2** was the demethyl form at C-24 of **1**. The relative stereochemistry of the tetracyclic part of **2** was the same as that of **1** from the NOESY data and $^1H-^1H$ coupling constants. Two hydroxyl groups at C-20 and C-22 were β- and α-oriented, respectively, judging from ^{13}C NMR chemical shifts of C-20 and C-22 (δ_C 76.8, s; 77.3, d) in **2**.[5] The CD curves of **2** showed positive Cotton effects, θ +8400 at 328 nm and θ −13 600 at 255 nm, suggesting that the absolute configuration of the tetracyclic portion of **2** was the same as that of **1**. The chemical shift differences of the (+)- and (−)-MTPA esters (**6a** and **6b**) revealed that the absolute configuration at C-25 of **2** was *R*.

This is the first isolation of ecdysteroids from a zoanthid of the genus *Palythoa*, although a few ecdysteroids have been isolated from the Mediterranean zoanthid *Gerardia savaglia*[8] and the Australian zoanthid *Parazoanthus* sp.[9] Ecdysteroids containing a hydroxyl group at C-26, such as **1** and **2**, have also been isolated from the terrestrial plant *Achyranthes fauriei*.[4]

Experimental Section

General Experimental Methods. UV and IR spectra were recorded on JASCO Ubest-35 and JASCO IR report-100 and FT/IR-230 spectrometers, respectively. Optical rotations were determined on a JASCO DIP-370 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX-500 spectrometer. The 7.19 and 123.5 ppm resonances of C_5D_5N were used as internal references. EIMS were obtained on a JEOL DX-303 spectrometer operating at 70 eV. FABMS were measured on a JEOL HX-110 spectrometer using a glycerol matrix.

Animal Material. The zoanthid *Palythoa australiae* Carlgren was collected off Seragaki, Okinawa Island, and kept frozen until used. The voucher specimen (ZT-1) has been deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Table 1. ^1H and ^{13}C NMR Data of Palythoalones A (**1**) and B (**2**) in C_5D_5N

	1			**2**		
position	$^1H^a$	*J* (Hz)	$^{13}C^a$	$^1H^a$	*J* (Hz)	$^{13}C^a$
1(a)	2.21 s		35.0 t	2.20 s		35.0 t
1(b)	2.10 m			2.13 m		
2	4.25 br d	11.8	68.0 d	4.25 br d	12.1	68.1 d
3	4.17 br s		69.9 d	4.16 br s		69.9 d
4(a)	2.13 m		36.1 t	2.08 m		36.1 t
4(b)	1.99 dd	14.7, 1.0		1.98 m		
5			80.0 s			79.9 s
6			200.9 s			200.8 s
7	6.28 d	2.1	119.8 d	6.27 d	2.5	119.9 d
8			166.9 s			166.8 s
9	3.65 m		38.4 d	3.63 m		38.4 d
10			44.9 s			44.9 s
11(a)	1.91 m		21.5 t	1.87 m		21.5 t
11(b)	1.83 m			1.80 m		
12(a)	2.18 m		31.7 t	2.18 m		
12(b)	1.81 m			1.98 m		31.8 t
13			48.2 s			48.2 s
14			84.0 s			84.0 s
15(a)	2.60 dt	4.7, 12.8	32.2 t	2.62 dt	5.1, 12.7	32.2 t
15(b)	2.03 m			2.05 m		
16(a)	2.44 m		22.2 t	2.45 m		22.2 t
16(b)	2.05 m			2.07 m		
17	2.93 t	9.0	49.9 d	2.95 m		50.0 d
18	1.22 s		18.0 q	1.22 s		18.0 q
19	1.16 s		17.3 q	1.16 s		17.2 q
20			76.8 s			76.8 s
21	1.58 s		21.6 q	1.59 s		21.5 q
22	3.99 d	10.3	74.1 d	3.85 d	10.8	77.3 d
23(a)	1.88 d	10.0	37.9 t	1.95 m		30.1 t
23(b)	1.54 m			1.65 m		
24	2.54 m		31.0 d	2.25 m		31.8 t
				1.41 m		
25	2.24 m		37.7 d	1.82 m		36.6 d
26(a)	3.79 dd	6.9, 10.2	66.8 t	3.78 dd	6.4, 10.2	67.4 t
26(b)	3.72 dd	6.9, 10.2		3.70 dd	6.4, 10.2	
27	0.92 d	6.9	10.9 q	1.02 d	6.4	17.3 q
28	0.91 d	6.8	15.9 q			

[a] δ in ppm.

Extraction and Isolation. The zoanthid *P. australiae* (1.0 kg, wet wt) was extracted with MeOH (600 mL × 2). The MeOH extract was partitioned between EtOAc (300 mL × 3) and H_2O (300 mL). The combined EtOAc-soluble portions were evaporated under reduced pressure to give a residue (2.6 g), part of which (1.0 g) was subjected to a Si gel column eluted with $CHCl_3$−MeOH (8:2) to give fractions A (51.1 mg) and B (97.5 mg). Fraction A was separated on a Sep-Pak ODS Cartridge (Waters) with MeOH−H_2O (8:2) to afford palythoalone A (**1**, 43.0 mg), while fraction B was separated by Si gel HPLC (YMC-Pack A-023, YMC Co., Ltd., 1.0 × 25 cm; flow rate 2.5 mL/min; UV detection at 254 nm; eluent $CHCl_3$−MeOH, 13:1) to afford palythoalone B (**2**, 6.0 mg, t_R 21.2 min), makisterone B (2.6 mg), and inokosterone (4.0 mg).

Palythoalone A (1): colorless amorphous solid; $[\alpha]^{26}_D$ +87° (*c* 2.2, MeOH); IR (film) ν_{max} 3435 and 1635 cm^{-1}; UV (MeOH) λ_{max} 243 nm (ϵ 10 000); CD (MeOH) 329 (θ +9100) and 254 (−14 000) nm; ^1H and ^{13}C NMR (Table 1); $^1H-^1H$ COSY correlations (C_5D_5N, H/H): 1/2, 2/3, 3/4, 9/11, 11/12, 15/16, 16/17, 22/23, 23/24, 24/28, 25/26, and 25/27; HMBC correlations (CD_3OD, H/C) 1a/10, 1a/19, 1b/5, 1b/9, 1b/10, 1b/19, 3/1, 3/5, 4/6, 7/5, 7/6, 7/9, 7/14, 9/1, 9/19, 11/10, 12/9, 12/13, 12/14, 15a/13, 15a/14, 15a/17, 16/13, 16/14, 16/20, 17/12, 17/13, 17/15, 17/18, 17/20, 17/21, 17/22, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 21/17, 21/20, 21/22, 22/17, 22/20, 22/21, 22/24, 23/25, 23/28, 24/25, 25/23, 25/24, 25/28, 26/24, 27/24, 27/26, 28/23, and 28/24; NOESY correlations (CD_3OD, H/H) 1a/19, 1b/19, 2/4a, 2/9, 4a/9, 7/15a, 7/15b, 9/12a, 11a/19, 11b/19, 12a/17, 12b/18, 12b/21, 15a/17, 15a/18, 16a/18, 17/21, 17/22, 17/23a, 18/21, 22/28, 26a/27, and 26b/27; FABMS *m*/*z* 511 $[M + H]^+$; HRFABMS *m*/*z* 511.3263 $[M + H]^+$, calcd for $C_{28}H_{47}O_8$, 511.3271.

Palythoalone B (2): colorless amorphous solid; $[\alpha]^{28}_D$ +56° (*c* 1.0, MeOH); IR (film) ν_{max} 3422 and 1654 cm^{-1}; UV (MeOH)

λ_{max} 245 nm (ϵ 11 000); CD (MeOH) 328 (θ +8400) and 255 (−13 600) nm; ^{1}H and ^{13}C NMR (Table 1); ^{1}H−^{1}H COSY correlations (C_5D_5N, H/H) 1/2, 2/3, 3/4, 9/11, 11/12, 15/16, 16/17, 22/23, 23/24, 25/26, and 25/27; HMBC correlations (C_5D_5N, H/C) 1a/2, 1a/3, 1a/10, 1a/19, 1b/2, 1b/3, 1b/5, 1b/9, 4a/3, 4a/5, 4a/10, 4b/2, 4b/3, 4b/5, 9/10, 9/11, 9/19, 11b/12, 12a/11, 12a/14, 12b/13, 15a/13, 15a/16, 15b/14, 15b/16, 16a/14, 16a/15, 16a/17, 17/13, 17/15, 17/16, 17/18, 17/21, 17/22, 18/12, 18/13, 18/14, 19/10, 21/17, 21/20, 22/20, 22/21, 22/24, 25/24, 27/24, and 27/26; NOESY correlations (C_5D_5N, H/H) 1a/19, 2/4a, 2/9, 3/4a, 3/4b, 4a/9, 9/11b, 11a/18, 11a/19, 11b/9, 12a/18, 16a/22, 16b/18, 16b/22, 17/21, 17/22, 18/21, 22/24b, 22/25, 22/26b, 23a/27, 24a/26a, 24a/27, 24b/27, 26a/27, and 26b/27; FABMS *m*/*z* 497 $[M + H]^+$; HRFABMS *m*/*z* 497.3118 $[M + H]^+$, calcd for $C_{27}H_{45}O_8$, 497.3115.

Absolute Stereochemistry at C-25 of 1 and 2. A solution of palythoalone A (**1**, 3.0 mg) and *p*-TsOH (0.24 mg) in 2,2-dimethoxypropane (0.2 mL) was stirred at room temperature for 4 h. $NaHCO_3$ (0.1 mg) was added to the reaction mixture, which was then extracted with $CHCl_3$ (3 mL × 3), and the $CHCl_3$ extract was evaporated to dryness. The residue was purified by a Si gel column ($CHCl_3$−MeOH, 19:1) to afford the 2,3- and 20,22-diacetonide derivative **3** (1.0 mg). Two aliquots of compound **3** (each 0.5 mg) were separately esterified with (+)−(*S*)- and (−)-(*R*)-MTPACl (each 3 μL) in dry pyridine (0.05 mL) under argon, followed by evaporation and a Si gel column chromatography ($CHCl_3$−MeOH, 39:1) to afford the (+)−(*R*)- and (−)-(*S*)-MTPA esters (**4a** and **4b**) of **3**, respectively.

According to essentially the same procedure as described above, the 2,3- and 20,22-diacetonide derivative **5** (1.0 mg) was derived from **2** (2.0 mg). Two aliquots of compound **5** (each 0.5 mg) were separately esterified with (+)−(*S*)- and (−)-(*R*)-MTPACl to afford the (+)−(*R*)- and (−)-(*S*)-MTPA esters (**6a** and **6b**) of **5**, respectively.

Compound 3: colorless amorphous solid; $[\alpha]^{26}_D$ +68° (*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3434 and 1674 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.83 (9H, m), 1.14 (3H, s), 1.25 (3H, s), 1.32 (3H, s) 1.34 (3H, d, J = 7.2 Hz), 1.41 (3H, s), 1.44 (1H, m), 1.55 (3H, s), 1.72 (3H, m), 1,79 (2H, m), 1.85 (3H, m), 2.06 (4H,m), 2.11 (2H, m), 2.17 (1H, m), 2.86 (1H, m), 3.54 (2H, m), 3.73 (1H, dd, J = 8.8, 2.4 Hz), 3.84 (1H, br s), 4.36 (2H, m), 5.93 (1H, d, J = 2.8 Hz); EIMS *m*/*z* 590 $[M^+]$ and 575 $[M - CH_3]^+$.

Compound 4a: ^{1}H NMR ($CDCl_3$) δ 0.85 (9H, m), 1.02 (3H, s), 1.25 (3H, s), 1.29 (3H, s), 1.34 (3H, s), 1.38 (3H, s), 1.49 (1H, m), 1.56 (3H, s), 1.72 (1H, m), 1.77 (2H, m), 1.79 (2H, m), 1.85 (2H, m), 2.01 (4H, m), 2,11 (3H, m), 2.18 (1H, m), 2,86 (1H, m), 3.48 (3H, s), 3.74 (1H, m), 4.20 (1H, m), 4.29 (1H, m), 4.35 (1H, m), 4.36 (1H, m), 4.39 (1H, m), 5.93 (1H, d, J = 2.8 Hz), 7.52 (5H, m); FABMS *m*/*z* 789 $[M - H_2O + H]^+$; HRFABMS *m*/*z* 789.4171 $[M - H_2O + H]^+$, calcd for $C_{44}H_{60}O_9F_3$, 789.4190.

Compound 4b: ^{1}H NMR ($CDCl_3$) δ 0.85 (9H, m), 1.11 (3H, s) 1.25 (3H, s), 1.28 (3H, s), 1.34 (3H, s), 1.39 (3H, s), 1.49 (1H, m), 1.56 (3H, s), 1.66 (1H, m), 1.72 (1H, m), 1.77 (2H, m), 1.79 (2H, m), 1.85 (3H, m), 2.03 (2H, m), 2.12 (2H, m), 2.18 (2H, m), 2.85 (1H, m), 3.55 (3H, s), 3.73 (1H, m), 4.04 (1H, m), 4.24 (2H, m), 4.37 (1H, m), 4.39 (1H, m), 5.94 (1H, d, J = 2.8 Hz), 7.52 (5H, m); FABMS *m*/*z* 807 $[M + H]^+$; HRFABMS *m*/*z* 807.4297 $[M + H]^+$, calcd for $C_{44}H_{62}O_{10}F_3$, 807.4296.

Compound 5: colorless amorphous solid; $[\alpha]^{26}_D$ +57°(*c* 0.1, $CHCl_3$); IR (film) ν_{max} 3444 and 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s), 0.89 (3H, s), 0.95 (3H, d, J = 6.4 Hz), 1.15 (3H, s), 1.26 (3H, s), 1.34 (3H, s), 1.41 (3H, s), 1.48 (1H, m), 1.56 (3H, s), 1.68 (2H, m), 1,78 (3H, m), 1.87 (3H, m), 2.06 (4H, m), 2.11 (2H, m), 2.20 (1H, m), 2.34 (1H, m), 2.86 (1H, m), 3.51 (2H, m), 3.64 (1H, m), 3.83 (1H, m), 4.36 (2H, m), 5.74 (1H, d, J = 2.4 Hz); EIMS *m*/*z* 576 $[M^+]$ and 561 $[M\text{-}CH_3]^+$.

Compound 6a: ^{1}H NMR ($CDCl_3$) δ 0.79 (3H, s), 0.88 (3H, s), 0.94 (3H, d, J = 6.4 Hz), 1.12 (3H, s), 1.31 (3H, s), 1.34 (3H, s), 1.40 (3H, s), 1.51 (1H, m), 1.52 (3H, s), 1.73 (1H, m), 1.78 (1H, m), 1.79 (1H, m), 1.86 (1H, m), 2.05 (6H, m), 2.11 (2H, m), 2.18 (2H, m), 2.34 (2H, m), 2.86 (1H, m), 3.55 (3H, s), 3.85 (1H, m), 4.05 (1H, m), 4.19 (1H, m), 4.31 (1H, m), 4.37 (1H, m), 4.40 (1H, m), 5.93 (1H, m), 7.52 (5H, m); FABMS *m*/*z* 793 $[M + H]^+$; HRFABMS *m*/*z* 793.4141 $[M + H]^+$, calcd for $C_{43}H_{60}O_{10}F_3$, 793.4139.

Compound 6b: ^{1}H NMR ($CDCl_3$) δ 0.79 (3H, s), 0.88 (3H, s), 0.96 (3H, d, J = 6.4 Hz), 1.11 (3H, s), 1.25 (3H, s), 1.31 (3H, s), 1.34 (3H, s), 1.49 (1H, m), 1.56 (3H, s), 1.72 (1H, m), 1.77 (2H, m), 1.79 (1H, m), 1.83 (1H, m), 1.99 (6H, m), 2.10 (2H, m), 2.17 (1H, m), 2.30 (1H, m), 2.37 (1H, m), 2.85 (1H, m), 3.49 (3H, s), 3.73 (1H, m), 4.12 (1H, m), 4.25 (2H, m), 4.37 (1H, m), 4.39 (1H, m), 5.93 (1H, m), 7.51 (5H, m); FABMS *m*/*z* 793 $[M + H]^+$; HRFABMS *m*/*z* 793.4157 $[M + H]^+$, calcd for $C_{43}H_{60}O_{10}F_3$, 793.4139.

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