New Cytotoxic Briaran Diterpenes from the Formosan Gorgonian *Briareum* sp.

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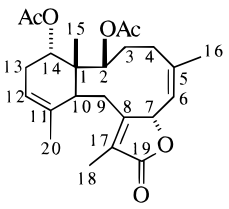
Three new briaran diterpenes, 2β-acetoxy-2-(debutyryloxy)stecholide E **(3)**, 9-deacetylstylatulide lactone **(4)**, and 4β-acetoxy-9-deacetylstylatulide lactone **(5)**, and two known diterpenes, brianthein W **(1)** and 9-deacetylbriareolide H **(2)**, have been isolated from a *Briareum* sp. of gorgonian. The structures and relative stereochemistry of these compounds were determined by spectroscopic and chemical methods. Diterpenes **2**−**4** and the corresponding 9-acetyl derivatives **6**, **7**, and **12** exhibited cytotoxicity toward various cancer cell lines.

Gorgonians (order Gorgonacea) and soft corals (Alcyonacea) are known to be important sources of diterpenoids in the marine environment. The gorgonacean genus *Briareum* has been the subject of many investigations that have led to the discovery of oxygenated diterpenes, including briareins,[1−11] asbestinins,[12−16] cladiellins,[7] and cembranes.[7] Briaran diterpenes continue to attract the attention of investigators because of the structural complexity and interesting bioactivity (e.g., cytotoxicity,[6,17,18] antiinflammatory,[9,19,20] antiviral,[6,20] insecticidal[3]) associated with several compounds of this type. The genus *Briareum* has been found to be situated near the transition between the Gorgonacea and Alcyonacea, both in chemical and taxonomic terms.[7,17] Previous studies have shown that species identification could be assisted by investigation of secondary metabolites from organisms of the *Briareum* sp.[2,5,8] In connection with our continuing studies of bioactive secondary metabolites from marine organisms, the chemistry of a specimen of *Briareum* sp., originally identified as *Pachyclavularia violacea* on the basis of similarity in colonial morphology, was studied. The specimen identification was subsequently revised on the basis of its chemical constituents.

Results and Discussion

In the present study, we have isolated brianthein W **(1)**[4] and 9-deacetylbriareolide H **(2)**,[9] which is identical with a reported briaran diterpene, (1*R*,2*R*,5*Z*,7*R*,8*S*,9*R*,10*R*,11*Z*,14*R*,17*S*)-2,14-diacetoxy-8,17-epoxy-9-hydroxybriara-5,11-dien-18-one,[21] in addition to three novel compounds 2β-acetoxy-2-(debutyryloxy)stecholide E **(3)**, 9-deacetylstylatulide lactone **(4)**, and 4β-acetoxy-9-deacetylstylatulide lactone **(5)** from the Formosan Gorgonian *Briareum* sp. The structures of these compounds were determined on the basis of spectroscopic and chemical methods. This report deals with the isolation, structure elucidation, and cytotoxicity of these compounds.

Briaran diterpenes **1**−**5** were isolated from Si gel column chromatography of the EtOAc-soluble fraction of *Briareum* sp. Compounds **2** and **3** were major metabolites, while **1**, **4**, and **5** were minor components.



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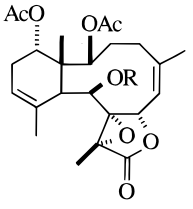
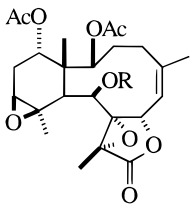


2 : R = H
6 : R = Ac



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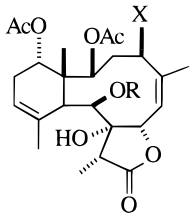
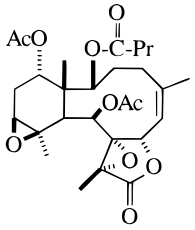


4 : R = H, X = H
5 : R = H, X = OAc
12 : R = Ac, X = H



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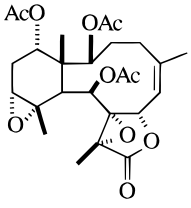

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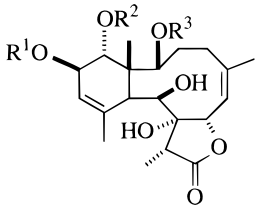
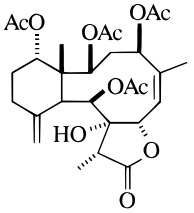


10 : R^1, R^2 = Ac, R^3 = n-C_5H_{11}CO
11 : R^1 = C_2H_5CO, R^2, R^3 = n-C_3H_7CO



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The spectral data (UV, IR, ^{1}H NMR, and MS) and melting point of **1** were in full agreement with those reported for a known diterpene, brianthein W.[4] However, the ^{13}C NMR spectrum of **1** (including DEPT experiments, see Table 2) showed that the chemical shift of C-9 appeared at δ 29.31, which is different from that

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Table 1. ^{1}H-NMR Chemical Shifts of Diterpenes **2**−**7** and **12**[a]

	compound						
H	**2**	**3**	**4**	**5**	**6**	**7**	**12**
2	4.87 d 5.9	4.84 d 6.3	4.92 d 6.6	4.82 d 6.6	5.03 d 6.6	4.88 d 6.6	4.98 d 7.8
3	2.87 m	2.76	2.92	3.32 dd	1.74 m	2.56 m	2.53 m
	1.66 m	dt 15; 5.1	dt 15.3; 3	14.4; 13.2		1.74 m	1.64 m
		1.61 m	1.52 m	1.90 m			
4	2.55 br d 13.6	2.51 br d 15.0	2.49 br d 15.3	5.04 dd 13.1; 5.4	2.56 m	2.54 br d 13.2	2.36 br s
	2.0 m	1.93 m	1.91 m			2.0 m	1.95 m
6	5.37 d 9.8	5.37 d 9.6	5.36 d 10.5	5.52 d 10.5	5.42 d 9.6	5.38 br s	5.37 br s
7	5.56 d 9.8	5.53 d 9.6	5.49 d 10.5	5.78 d 10.5	5.37 d 9.6	5.38 br s	5.37 br s
9	4.32 br s	4.41 br s	4.55 dd 6.3; 3.0	4.62 dd 6.9; 3.9	5.70 d 3	5.74 d 3.3	5.99 br s
10	2.74 br s	2.31 br s	2.65 br s	2.67 br s	2.84 m	2.44 d 3.3	2.81 br s
12	5.35 br s	3.07 d 4.5	5.39 d 4.8	5.45 d 5.1	5.42 m	3.02 d 5.1	5.44 br s
13	2.32 br d 19.0	2.10 m	2.31 br d 19.2	2.30 br d 18.0	2.21 m	2.03 m	2.0 m
	2.0 m		2.0 m	2.0 m	2.06 m		
14	4.78 br s	4.69 br s	4.79 br s	4.80 br s	4.74 br s	4.70 br s	4.77 br s
15	1.23 s	1.15 s	1.17 s	1.19 s	1.03 s	1.0 s	0.98 s
16	1.95 s	2.01 s	1.94 s	1.95 s	2.03 s	2.05 s	2.01 s
17			3.16 q 7.2	3.20 q 7.0			2.53 q 7.0
18	1.61 s	1.65 s	1.21 d 7.2	1.25 d 7.0	1.57 s	1.68 s	1.25 d 7.0
20	1.72 s	1.36 s	1.82 s	1.84 s	1.85 s	1.42 s	1.97 s
acetate	2.03 s	2.03 s	1.98 s	1.95 s	1.97 s	2.00 s	1.97 s
methyls	2.02 s	1.99 s	2.01 s	2.05 s	2.04 s	2.02 s	2.03 s
				2.05 s	2.17 s	2.17 s	2.17

[a] The chemical shifts were determined at 300 MHz in $CDCl_3$. The values are in ppm downfield from TMS.

Table 2. ^{13}C-NMR Chemical Shifts of Diterpenes **1**,[a] **2**−**7**, and **12**

	compound							
position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**12**
C-1	41.56	45.06	45.39	44.74	45.36	44.73	45.17	44.41
C-2	74.51	75.31	75.41	75.53	73.49	74.87	74.95	74.66
C-3	33.79	32.17	32.49	31.46	37.38	32.50	32.77	31.82
C-4	29.40	28.69	28.85	28.74	72.58	26.98	28.80	28.64
C-5	143.06	146.14	146.20	147.45	146.08	146.18	146.29	148.05
C-6	123.74	118.43	118.17	117.59	122.19	118.15	118.19	117.03
C-7	80.28	74.73	74.44	78.42	77.42	74.55	74.43	78.46
C-8	159.16	71.56	71.27	83.00	82.63	70.69	70.61	81.69
C-9	29.31	70.83	72.15	71.71	71.55	68.80	70.07	70.24
C-10	37.71	44.22	42.32	41.33	41.10	43.59	42.11	40.26
C-11	136.90	134.64	64.05	135.46	135.05	133.46	63.54	134.34
C-12	116.75	120.24	61.50	120.42	120.80	120.74	60.77	120.74
C-13	26.47	26.48	25.21	26.78	26.49	28.63	25.11	26.62
C-14	72.42	74.25	73.88	73.96	73.16	73.48	73.78	73.38
C-15	14.60	16.53	15.77	15.81	15.92	15.10	15.29	14.10
C-16	27.48	26.96	27.27	27.64	25.97	26.32	27.30	27.64
C-17	124.90	62.34	62.56	43.95	43.76	63.35	62.60	43.73
C-18	9.67	9.44	9.37	7.00	6.91	9.74	10.03	7.05
C-19	173.28	172.22	170.71	177.89	176.87	170.54	170.58	175.91
C-20	21.57	24.56	24.45	24.53	24.76	24.47	24.40	24.31
acetate	20.75	21.17	21.10	21.20	21.12	21.17	21.08	21.16
methyls	20.88	21.31	21.10	21.44	21.15	21.32	21.11	21.44
					21.37	21.43	21.44	21.47
ester	170.13	170.65	170.71	170.66	170.32	168.87	167.42	169.62
carbonyls	170.31	171.23	172.05	171.42	170.51	171.09	170.61	170.49
					171.08	171.20	171.03	171.15

[a] The chemical shifts of **1** were determined at 100 MHz in C_6D_6. The ^{13}C-NMR spectra of other compounds were measured at 75 MHz in $CDCl_3$. The values are in ppm downfield from TMS.

reported previously (δ 53.43).[4] The absence of any methylene carbon of **1** resonating around 53 ppm suggests the need for revision of the data reported previously. Spectral data of compound **2** established a molecular formula, $C_{24}H_{32}O_8$. This compound was further identified as 9-deacetylbriareolide H[21] by comparison of its spectral data, mp, and optical rotation[22] with those of the known diterpene. Acetylation of **2** gave its acetate, briareolide H **(6)**,[9] and further confirmed the structure of **2**.

The new briaran diterpene **3** was the most abundant metabolite. Its mass spectrum established the composition $C_{24}H_{32}O_9$, and its other spectral data (IR, ^{1}H NMR, and ^{13}C NMR) indicated that it was closely related to **2** and was most probably an epoxide of **2**. Acetylation of **3** gave the less polar product **7**, which had ^{1}H and ^{13}C spectra nearly identical with those of stecholide E acetate **(8)**,[18] except that the signals for the butyrate group in **8** were replaced by those for an acetate group in **3**. The stereochemistry of the 11,12-epoxide was established as β by the fact that ^{13}C NMR shifts of C-11 and C-12 in **7** are significantly different from those of the isomer α-epoxide **9**.[9] The structure of **3** was thus established as 2β-acetoxy-2-(debutyryloxy)stecholide E.

The new briaran diterpene **4** had a molecular formula of $C_{24}H_{34}O_8$ as determined by HRMS. On the basis of its spectral data (IR, ^{1}H NMR, and ^{13}C NMR) and by comparison of these data with those of cavernuline

Table 3. Cytotoxicity of Diterpenes **1**−**7**, and **12**[a]

	cell lines ED_{50} (μg/mL)			
compd	P-388	KB	A-549	HT-29
1	0.76	>50	>50	>50
2	0.28	0.27	10.35	8.27
3	0.61	>50	>50	6.96
4	1.12	>50	>50	1.79
5	>50	>50	>50	>50
6	3.10	>50	>50	0.29
7	1.59	24.45	17.39	10.07
12	0.51	>50	>50	15.33

[a] For significant activity of pure compounds, an ED_{50} value of ≤4.0 μg/mL is required. See Geran *et al.*[27]

(10),[23] cavernulinine **(11)**,[23] and stylatulide lactone **(12)**,[24] diterpene **4** was identified as 9-deacetylstylatulide lactone. The structure of **4** was confirmed by acetylation to give a less polar product, which was found to be identical with stylatulide lactone **(12)**[24] by comparison of physical ($[\alpha]_D$) and spectral data.

The new briaran diterpene **5** had the composition $C_{26}H_{36}O_{10}$ as determined by HRMS. The spectral data (IR, ^{1}H NMR, and ^{13}C NMR) of **5** were similar to those of **4**, except that **5** showed signals corresponding an additional acetoxy substituent. The additional acetoxy-bearing methine proton gave a signal at δ 5.04 (1H, dd, J = 13.1, 5.4 Hz) and was assigned as H-4 based on a comparison with the ^{1}H NMR spectrum of the structurally related compound junceellolide D **(13)**.[20] On the basis of all the spectral data and by comparsion of these data with those of stylatulide lactone **(12)**,[24] 9-deacetylstylatulide lactone **(4)**, and junceellolide D **(13)**,[20] compound **5** was found to be 4β-acetoxy-9-deacetylstylatulide lactone with the relative stereochemistry as described by formula **5**.

The cytotoxicities of the briareins against the growth of P-388, KB, A-549, and HT-29 cells were studied and are shown in Table 3. These data show that all the briareins except compound **5** exhibited significant activities against the growth of P-388 cells. The cytotoxicities of 9-hydroxybriareins **2**, **3**, and **4** did not show significant difference in comparsion with their corresponding 9-acetyl derivatives **6**, **7**, and **12**. Compound **1** was inactive against the growth of KB, A-549, and HT-29 cells, indicating that oxidation at C-9, either in hydroxyl or in acetoxyl form, could increase the activity of briareins. Compound **5** was not cytotoxic to the above cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. Ultraviolet spectra (in ethanol) were recorded on a Hitachi U-3210 UV spectrophotometer and IR spectra on a Hitachi I-2001 infrared spectrophotometer. The NMR spectra were recorded on a VXR-300/5 FT- NMR at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Unity Plus 400 MHz FT-NMR for ^{1}H and 100 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard, unless otherwise indicated. EI and FABMS spectra were obtained with a VG QUATTRO GC/MS spectrometer. HREIMS and HRFABMS spectra were recorded on a JMX-HX 110 mass spectrometer. Silica gel (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Silical gel 60 F254, 0.2 mm) were used for analytical TLC.

Marine Organism. The organism *Briareum* sp. was collected by hand using scuba at the South Bay, Kenting, located in the southernmost tip of Taiwan in Oct 1991, at a depth of 5 m, and was stored in a freezer until extraction. The *Briareum* sp. is a species with encrusting, purplish, sheet-forming colonies that overgrow and cover substratum, most of which were dead corals. A voucher speciman was deposited in the Department of Marine Resources, National Sun Yat-Sen University (specimen no. KTSC-101).

Extraction and Separation. The marine organism (4.24 kg fresh wt) was collected and freeze-dried. The freeze-dried material (970 g) was minced and extracted exhaustively with ethyl acetate (8 L × 6). The organic extract was evaporated to give a dark-green residue (34.5 g). The ethyl acetate solution of the residue was stored at 0 °C to give a solid (3.5 g) that was found to be the mixture of long-chained esters formed from saturated fatty acids and alcohols and was discarded. The remaining mixture was concentrated and was triturated with hexane to yield a hexane-soluble fraction (17.4 g), which was found to be the mixture of low polar cembrenes, steroids, and fatty acids. The remaining hexane-insoluble but ethyl acetate-soluble fraction (13.2 g) was separated by Si gel column chromatography, using hexane and hexane−EtOAc mixtures of increasing polarity. Diterpene **1** was eluted with hexane−EtOAc (4:1), **2** with hexane−EtOAc (3:1), **3** with hexane−EtOAc (3:1 → 2:1), **4** with hexane−EtOAc (2:1 → 3:2), and **5** with hexane−EtOAc (3:2).

Brianthein W (1): colorless crystals (46 mg); mp 207−209 °C (lit.[4] mp 205−209 °C); $[\alpha]^{24}_D$ +62° (*c* 0.45, $CHCl_3$); UV (EtOH) λ_{max} 230 nm (ϵ 7800); IR (KBr) ν_{max} 1750, 1732, 1668, 1374, 1256 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.49 (1H, br d, J = 8.7 Hz), 5.22 (1H, d, J = 6 Hz), 5.16 (1H, d, J = 9.3 Hz), 4.89 (1H, br s), 4.84 (1H, br s), 2.88 (1H, br d, J = 15.9 Hz), 2.69 (1H, m), 2.58 (1H, m), 2.52 (1H, dd, J = 15.9, 7.5 Hz), 2.35−2.10 (2H, overlapping m), 2.06 (3H, s), 2.03 (3H, s), 1.95 (3H, s), 1.89 (3H, s), 1.60 (3H, s), 1.01 (3H, s); ^{13}C NMR data in Table 2; FABMS *m/z* (relative intensity) 417 [10.1, $(M + H)^+$], 391 (7.4), 357 (1.0, $M^+ - OAc$), 307 (5.2), 297 (4.1), 289 (4.5).

9-Deacetylbriareolide H (2): colorless crystals (150 mg); mp 239−241 °C (lit.[21] 240−241 °C); $[\alpha]^{26}_D$ +13° (*c* 0.32, $CHCl_3$) (lit.[21] $[\alpha]^{26}_D$ +2.3°); IR (KBr) ν_{max} 3420, 1776, 1732, 1712, 1458, 1258, 1032 cm^{-1}; ^{1}H and ^{13}C NMR data in Tables 1 and 2; EIMS (30 eV) *m/z* (relative intensity) 388 (0.9, $M^+ - AcOH$), 328 (2.2, $M^+ - 2AcOH$), 313 (3.0), 256 (3.6), 183 (23.3), 133 (50.7), 119 (100), 107 (65.0), 81 (30.0).

2β-Acetoxy-2-(debutyryloxy)stecholide E (3): colorless crystals (194 mg); mp 218−220 °C; $[\alpha]^{26}_D$ +27° (*c* 1.2, $CHCl_3$); IR (KBr) ν_{max} 3428, 1778, 1734, 1714, 1250, 970 cm^{-1}; ^{1}H and ^{13}C NMR in Tables 1 and 2; FABMS *m/z* (relative intensity) 465 [3.6, $(M + H)^+$], 447 (2.5, $M^+ - OH$), 405 (1.6, $M^+ - OAc$), 345 (1.5), 307 (3.8), 289 (3.5), 257 (2.8); HRFABMS *m/z* 465.2113, calcd for $C_{24}H_{33}O_9$ 465.2115.

9-Deacetylstylatulide lactone (4): colorless crystals (36 mg); mp 229−231 °C; $[\alpha]^{26}_D$ −3° (*c* 0.58, $CHCl_3$); IR (KBr) ν_{max} 3472,1780,1748, 1735, 1374, 1272, 1214, 1034 cm^{-1}; ^{1}H and ^{13}C NMR data in Tables 1 and 2; FABMS

m/z (relative intensity) 451 [0.7, $(M + H)^+$], 433 (0.5, $M^+ - OH$), 391 (2.1, $M^+ - OAc$), 331 (0.9), 307 (9.3), 289 (4.9), 257 (1.3); HRFABMS *m/z* 451.2315, calcd for $C_{24}H_{35}O_8$ 451.2322.

4β-Acetoxy-9-deacetylstylatulide lactone (5): colorless crystals (2.7 mg); mp 216−217 °C; $[\alpha]^{26}_D$ +44° (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 3480, 1775, 1740, 1360, 1250, 1024 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; EIMS (70 eV) *m/z* (relative intensity) 508 (0.2, M^+), 449 (0.2, $M^+ - OAc$), 388 (0.6, $M^+ - 2AcOH$), 328 (1.5), 299 (0.9), 227 (2.8), 132 (16.7), 119 (26.4), 107 (22.2), 43 (100); HREIMS *m/z* 508.2349, calcd for $C_{26}H_{36}O_{10}$ 508.2298.

Briareolide H (6). 9-Deacetylbriareolide H **(2)** (12.6 mg) was stirred with 3 mL of acetic anhydride in 3 mL of pyridine for 48 h at room temperature. After evaporation of excess reagent, the residue was separated by column chromatography, on silica gel (hexane−EtOAc, 5:1), to give pure briareolide H **(6)** (6.9 mg, 50%) as colorless crystals: mp 205−207 °C; $[\alpha]^{26}_D$ −6° (*c* 0.22, $CHCl_3$); IR (KBr) ν_{max} 2956, 1780, 1732, 1374, 1248, 1218, 1040 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; FABMS *m/z* (relative intensity) 491 [0.6, $(M + H)^+$], 431 (1.1, $M^+ - OAc$), 391 (1.8), 371 (1.1), 311 (1.9), 241 (4.4), 223 (9.8).

2β-Acetoxy-2-(debutyryloxy)stecholide E Acetate (7). According to the above procedure, 2β-acetoxy-2-(debutyryloxy)stecholide E **(3)** (18.7 mg) was acetylated to give the product 2β-acetoxy-2-debutyryloxystecholide E acetate **(7)** (15.1 mg 74%) as colorless crystals: mp 211−213 °C; $[\alpha]^{26}_D$ −5° (*c* 0.55, $CHCl_3$); IR (KBr) ν_{max} 2996, 1782, 1768, 1738, 1374, 1250, 1222 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; FABMS *m/z* (relative intensity) 507 [6.8, $(M + H)^+$], 447 (1.1, $M^+ - OAC$), 387 (0.6), 327 (0.9), 307 (6.3), 289 (4.7); HRFABMS *m/z* 507.2232, calcd for $C_{26}H_{35}O_{10}$ 507.2220.

Stylatulide Lactone (12). According to the above procedure, 9-deacetylstylatulide lactone **(4)** (10.6 mg) was acetylated to give the product stylatulide lactone **(12)** (7 mg, 60%) as colorless crystals: mp 107−109 °C; $[\alpha]^{26}_D$ −7° (*c* 0.1, $CHCl_3$) (lit.[24] $[\alpha]^{26}_D$ −7°); IR (KBr) ν_{max} 3452, 1770, 1744, 1716, 1376, 1250, 1200 cm^{-1}; ^1H and ^{13}C NMR data in Tables 1 and 2; EIMS (30 eV) *m/z* (relative intensity) 432 (0.5, $M^+ - AcOH$), 414 (0.2, $M^+ - AcOH - H_2O$), 372 (6.0, $M^+ - 2AcOH$), 330 (28.5), 312 (12.0), 256 (15.5), 211 (23.4), 167 (59.9), 133 (87.6), 119 (100).

Cytotoxicity Testing. KB and P-388 cells were kindly provided by Prof. J. M. Pezzuto, University of Illinois at Chicago; A-549 (human lung adenocarcinoma) and HT-29 (human colon adenocarcinoma) were purchased from the American Type Culture Collection.

Cytotoxicity assays were carried out according to the procedure described previously.[25,26]

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