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New polyoxygenated steroids exhibiting reversal of multidrug resistance from the gorgonian *Isis hippuris*

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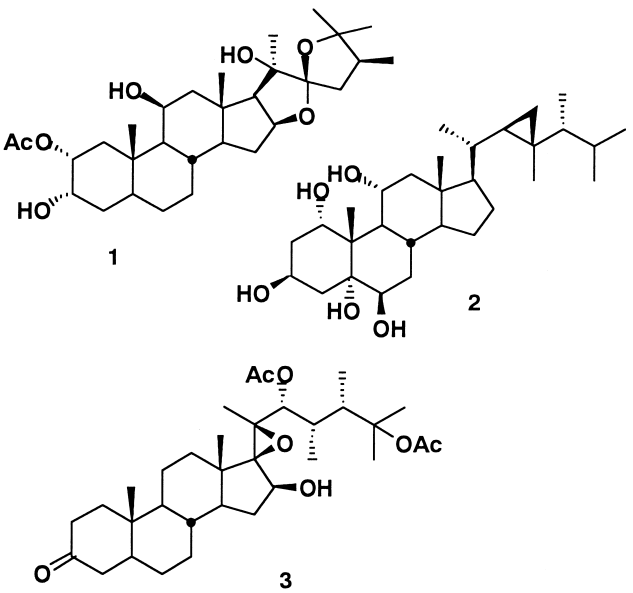
Abstract—Eleven new (**5**–**15**) and one known (**4**) polyoxygenated steroids have been isolated from two collections of the gorgonian *Isis hippuris* and their structures determined by spectroscopic analysis and X-ray diffraction study. Except for **15** all others were polyoxygenated gorgosterols. X-Ray study of the previously reported compound **4** demonstrated the stereochemistry of the side chain moiety to be identical with that of gorgosterol. Compound **15** was a deacetyl derivative of the known steroid **16** having a spiroketal and a lactone function. The stereochemistry of **15** was confirmed by X-ray analysis, which also established the C-22 configuration of **16** which had remained unsolved. These compounds have been tested for reversal of multidrug resistance (MDR) with cancer cells expressing P-glycoprotein (P-gp). Some of them showed moderate activity.

1. Introduction

A large number of uncommon steroids have been described from marine sources.[1] A dominant feature is the wealth of polyoxygenated structures found mainly from echinoderms, sponges, and octocorals.[2] Another characteristic of marine steroids is the variation in side chain alkylation. An example is gorgosterol, the first cyclopropane-containing sterol. The structure of gorgosterol was solved in a cooperative effort by members of several laboratories including the Hawaii group in 1970.[3] Since then sterols having a various side chain alkylation have been reported mainly from sponges.[4]

The gorgonian *Isis hippuris* is unique in its steroidal constituents: structures having both polyoxygenation and the side chain variation have been reported. Since the first report of hippurin-1 (**1**),[5] the gorgonian has been subject of research by several groups and yielded a number of polyoxygenated steroids. Structures of these compounds can be characterized as hippurin or hippuristanol type (e.g. **1**) having a spiroketal,[6–8] gorgosterol type (e.g. **2**) possessing a cyclopropane,[8,9] and hippuristerone type (e.g. **3**) containing a 3-keto function.[10,11] These types also differ in the side chain alkylation pattern. Some hippuristanols have been reported to have significant antitumor activity,[7] while no biological activity has yet been described for the other two types. In view of the recent reports of fascinating activity of such polyoxygenated steroids as the antitumor aragusterols[12,13] and multidrug resistance modulating agosterols,[14,15] we undertook reinvestigation of the steroidal constituents of *I. hippuris* and isolated eleven new and one known polyoxygenated steroids. In this paper we describe the isolation, structure elucidation, and biological activity of these compounds.



Keywords: octocoral; gorgosterols; hippuristanols; X-ray analysis.

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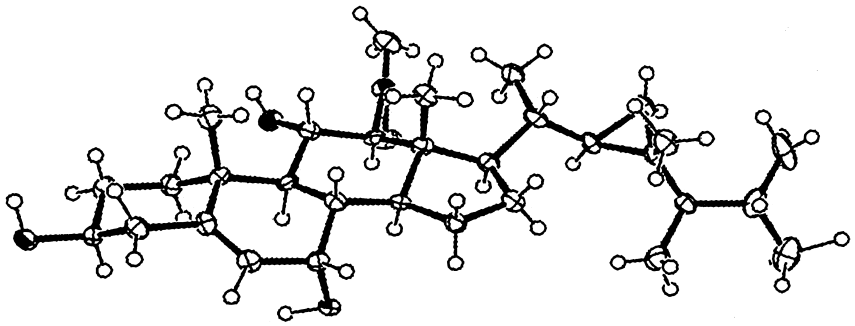


Figure 1. Computer-generated ORTEP drawing of compound **4**.

2. Results and discussion

2.1. Isolation and structures

Two collections of *I. hippuris* were employed in the present study. One specimen from the island of Okinawa yielded two steroids **4** and **7**, while the other sample from the island of Yonaguni yielded 10 compounds (**5**, **6**, **8**–**15**).

Sterol **4** was described in our earlier report.[9] However, the stereochemistry of the side chain remained unsolved. Fortunately, a sample of **4** could be isolated as crystals in the present work and analyzed by single crystal X-ray diffraction. The result (Fig. 1) confirmed the overall structure of **4** and revealed the side chain stereochemistry which was identical with that of gorgosterol.[3] All of its NMR signals have also been assigned for the first time as shown in Tables 1 and 2.

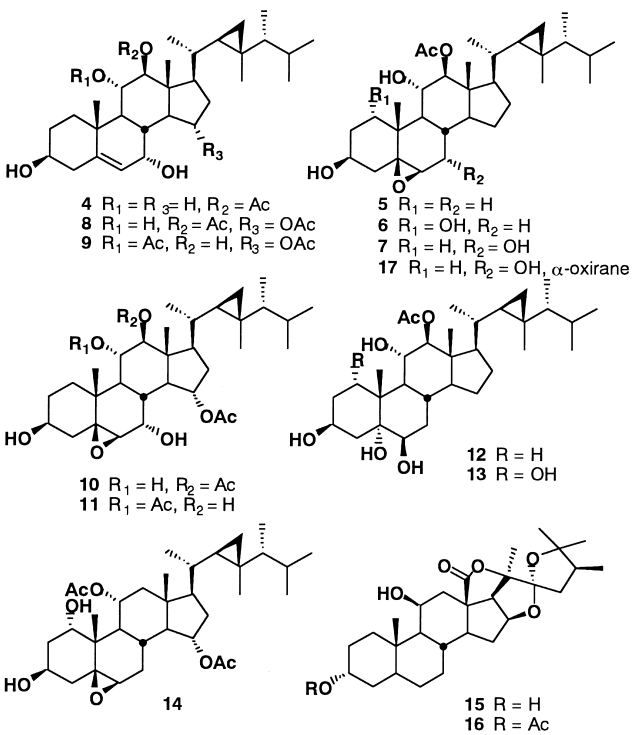
Table 1. ^{1}H NMR data for steroids **4**–**9** in $CDCl_3$

No.	4	5	6	7	8	9
1	a1.16 dt	a1.22 m	4.27 t	a1.40 m	1.23 m	b1.62 dt
	b2.58 dt	b2.60 dt		b2.60 brd	2.52 dt	a1.32 dt
2	a1.53 m	a1.75 dt	a1.70 dt	a1.40 m	1.53 m	a1.49 brd
	b1.77 brd	b1.42 dt	b2.10 dt	b1.72 m	1.81 dt	b1.81 m
3	3.45 tt	3.67 m	4.16 m	3.60 m	3.61 m	3.62 m
4	a2.26 dd	a1.39 m	1.58 m	a1.36 m	2.24 dd	a2.41 dd
	b2.34 ddd	b2.09 m	2.01 dd	b2.06 m	2.38 m	b2.24 dd
6	5.60 brd	3.09 d	3.11 d	3.10 s	5.60 d	5.60 dd
7	3.95 brs	a1.29 t	a1.37 dd	4.15 brs	3.88 brs	3.89 m
	–	b2.07 m	b2.06 dt	–	–	–
8	1.42 dt	1.62 m	1.60 m	1.62 m	1.59 m	1.61 m
9	1.60 t	0.85 t	1.79 t	1.43 m	1.66 dd	1.95 dd
11	3.80 brq	3.75 td	3.79 t	3.70 brq	3.85 dd	5.19 dd
12	4.72 d	4.65 d	4.67 d	4.60 d	4.82 d	3.48 dd
14	1.69 dt	1.11 m	1.22 m	1.47 m	1.98 dd	1.81 m
15	a1.26 m	a1.18 m	1.21 m	a1.17 m	4.85 dt	4.87 dd
	b1.90 m	b1.65 m	1.71 m	b1.83 m	–	–
16	a1.60 m	a1.59 m	1.58 m	a1.59 m	1.82 m	1.78 dt
	b2.08 m	b1.93 m	1.93 m	b2.02 m	2.40 m	2.40 m
17	1.58 m	1.53 m	1.55 m	1.52 m	1.87 m	1.80 m
18	0.82 s	0.80 s	0.78 s	0.78 s	0.98 s	0.83 s
19	1.12 s	1.17 s	1.15 s	1.16 s	1.10 s	1.11 s
20	1.12 m	1.13 m	1.18 m	1.11 m	1.20 m	1.22 m
21	0.95 d	0.91 d	0.91 d	0.90 d	0.94 d	1.11 d
22	0.18 dt	0.19 dt	0.21 dt	0.18 m	0.19 dt	0.36 dt
24	0.20 m	0.24 td	0.24 td	0.21 m	0.24 dq	0.27 dq
25	1.56 m	1.57 m	1.56 m	1.56 m	1.54 m	1.59 m
26	0.85 d	0.86 d	0.86 d	0.85 d	0.86 d	0.93 d
27	0.93 d	0.94 d	0.94 d	0.92 d	0.91 d	0.86 d
28	0.92 d	0.92 d	0.92 d	0.90 d	0.94 d	0.94 d
29	0.91 s	0.90 s	0.90 s	0.89 s	0.91 s	0.92 s
30	−0.10 dd	−0.11 dd	−0.10 dd	−0.10 brt	−0.07 brt	−0.09 dd
	0.43 dd	0.46 dd	0.47 dd	0.42 dd	0.48 dd	0.05 dd
Ac	2.11 s	2.10 s		2.12 s	2.10 s	2.03 s
	–			–	2.11 s	2.05 s
7-OH					2.55 brd	2.47 d
12-OH						2.24 d
OH			3.00 br			

Table 2. ^{13}C NMR data for steroids **4**–**9** in $CDCl_3$

No.	4	5	6	7	8	9
1	38.3 t	38.7 t	73.9 d	38.6 t	38.2 t	37.2 t
2	31.5 t	31.1 t	38.0 t	31.2 t	31.3 t	31.5 t
3	71.0 d	69.3 d	63.9 d	69.0 d	71.0 d	70.6 d
4	41.9 t	42.6 t	42.3 t	42.2 t	42.2 t	42.3 t
5	145.7 s	63.1 s	62.8 s	64.3 s	146.0 s	145.8 s
6	123.1 d	63.0 d	63.9 d	64.4 d	120.0 d	122.7 d
7	65.1 d	31.6 t	32.4 t	67.4 d	64.4 d	64.5 d
8	36.5 d	28.0 d	27.6 d	33.1 d[a]	36.6 d	36.7 d
9	48.8 d[a]	56.9 d	47.9 d	48.4.d	48.2 d	44.9 d
10	38.8 s	35.9 s	40.9 s	35.4 s	39.0 s	38.9 s
11	72.4 d	73.2 d	73.1 d	73.0 d	72.8 d	76.7 d
12	84.6 d	85.1 d	87.1 d	84.9 d	84.3 d	82.4 d
13	46.9 s	47.0 s	46.9 s	46.9 s	46.8 s	47.9 s
14	48.3 d[a]	53.7 d	52.9 d	48.6 d	51.1 d	50.5 d
15	23.7 t	23.6 t	23.8 t	23.1 t	76.6 d	76.9 d
16	28.4 t	27.7 t	27.5 t	28.3 t	37.0 t	37.3 t
17	58.2 d	57.6 d	57.6 d	58.2 d	55.1 d	55.6 d
18	9.5 q	10.0 q	9.9 q	9.7 q	10.9 q	10.1 q
19	17.6 q	15.6 q	15.9 q	16.0 q	17.7 q	18.0 q
20	33.5 d	33.2 d	33.0 d	33.2 d[a]	32.5 d	32.7 d
21	22.4 q	22.3 q	22.6 q	22.6 q	22.1 q	22.7 q
22	30.9 d	30.3 d	30.0 d	30.7 d	29.7 d	29.8 d
23	25.7 s	25.3 s	25.2 s	25.7 s	25.2 s	25.3 s
24	50.5 d	50.6 d	50.6 d	50.6 d	50.5 d	50.4 d
25	32.1 d	32.1 d	32.1 d	32.2 d	32.0 d	32.1 d
26	21.5 q	21.4 q	21.4 q	21.5 q	21.4 q	21.5 q
27	22.2 q	22.2 q	22.2 q	22.3 q	22.4 q	22.2 q
28	15.1 q	15.4 q	15.4 q	15.2 q	15.3 q	15.1 q
29	13.9 q	13.8 q	13.8 q	13.9 q	13.8 q	13.7 q
30	21.3 t	21.3 t	21.3 t	21.4 t	21.1 t	21.3 t
Ac	21.9 q	21.7 q	21.8 q	22.0 q	21.6 q	21.2 q
					21.6 q	21.8 q
	173.3 s	173.2 s	173.9 s	173.7 s	170.1 s	170.0 s
					172.5 s	173.1 s

[a] Signals in the same column may be exchanged.



Compound **5** was isolated as a glass. The same molecular formula $C_{32}H_{52}O_5$ as **4** was deduced from HREIMS measurement on a fragment ion at *m/z* 456 ($[M-AcOH]^+$, $\Delta=-2.1$ mmu). The NMR spectra indicated the presence of the same functionality: 3β-OH (δ 3.67 m; δ 69.3 d), 11α-OH and 12β-OAc (δ 3.75 td, 4.65 d, 2.10 s; δ 73.2 d, 85.1 d, 21.7 q, 173.2 s; 1720 cm^{-1}), and the cyclopropyl side chain (δ −0.11 dd, 0.19 dt, 0.24 td, 0.46 dd) except for an epoxy group (δ 3.09 d; δ 63.1 s, 63.0 d) in place of a double bond in **4**. The position of the oxirane was determined to be at C-5 and C-6 by COSY (H-6/H-4β,H-7β) and HMBC crosss peaks (H-4αβ/C-5, H-6/C-7,8, H-7β/C-6, H-19/C-5). Close similarity of the ^{13}C NMR data for rings A and B portions [δ 69.3 (C-3), 42.6 (C-4), 63.1 (C-5), 63.0 (C-6)] of **5** to those [δ 69.4 (C-3), 42.2 (C-4), 63.0 (C-5), 63.7 (C-6)] of 5β,6β-epoxycholestan-3β-ol but not to those [δ 68.5 (C-2), 39.9 (C-4), 66.0 (C-5), 59.5 (C-6)] of 5α,6α-epoxycholestan-3β-ol strongly supported the β-orientation of the oxirane.[16] Furthermore, NOE observation between H-6/H-3,H-4α,H-7α reinforced the above conclusion.[17] Thus, the structure is 5β,6β-epoxygorgostane-3β,11α,12β-triol 12-acetate.

The formula of compound **6** $C_{32}H_{52}O_6$ as deduced from HREIMS contained an additional oxygen atom as compared to **5**. It was shown to have an additional hydroxyl group at C-1 (δ 4.27 t; δ 73.9 d) by COSY (H-1/H-2αβ, H-3/H-2αβ) and HMBC cross peaks (H-19/C-1). The orientation of the hydroxyl group was determined to be α as evidenced by a small coupling constant of H-1 (t, $J=3$ Hz) and NOE observed for H-1/H-2β and H-19. Except for ring A, C-9, and C-10 portions, the NMR signals were almost identical with those of **5**. Thus, the structure of **6** could be deduced as 5β,6β-epoxygorgostane-1α,3β,11α,12β-tetrol 12-acetate.

Compound **7**, $C_{32}H_{52}O_6$ ($\Delta=+0.1$ mmu for $[M-H_2O]^+$), showed nearly identical NMR signals to those of **5** except for the resonances corresponding to C-7–C-9 and C-14. As in the case of **6**, **7** has an additional hydroxyl group which can be placed at C-7 of **5** by COSY [H-7 (δ 4.15)/H-6,H-8] and HMBC [C-7 (δ 67.4 d)/H-6)]. The α-orientation of the 7-OH was shown by the signal shape of H-7 (brs) and NOE of H-7/H-8,H-15αβ. The configuration of the epoxy moiety was shown to be 5β,6β (δ 3.10 d; δ 64.3 s, 64.4 d) by similar NMR chemical shifts for ring A portion with those of **5** and NOE of H-6/H-3.[17] Comparison with 5α,6α-epoxy derivative (**17**) prepared by MCPBA oxidation[16] of **4** reinforced the stereochemical assignment of the epoxy moiety in **7**. The signals for **17** (δ 3.25 d; δ 69.4 s, 62.8 d) were different from those of **7** but similar to those reported for 5α,6α-epoxysteroids.[16,18]

Spectral data of compound **8**, ($C_{34}H_{54}O_7$, $\Delta=-0.2$ mmu for $[M-2H_2O]^+$), indicated the presence of a double bond (δ 5.60 d; δ 146.0 s, 120.0 d), two acetoxy groups (δ 2.11 s, 2.10 s; δ 172.5 s, 170.1 s, 21.6 q×2; 1729 cm^{-1}), and five oxymethines (δ 4.85 dt, 4.82 d, 3.88 brs, 3.85 dd, 3.61 m; δ 84.3, 76.6, 72.8, 71.0, 64.4; 3417 cm^{-1}). Comparison of the NMR data with those of **4** and analysis of 2D NMR data suggested that **8** had an additional acetoxy group which could be placed at C-15 (δ 4.85 dt; δ 76.6 d). The orientation of the 15-OAc was shown to be α by NOE (H-15/H-18) observation.

Table 3. ^{1}H NMR data for steroids **10**–**15** in $CDCl_3$

No.	**10**	**11**	**12**[a]	**13**[a]	**14**	**15**
1	a1.36 dt	a1.79 m	a1.96 m	4.04 brs	3.96 dd	a1.43 m
	b2.53 brd	b1.45 dt	b1.77 dt	–	–	b1.72 m
2	a1.43 brt	a2.17 m	a1.48 m	a2.05 m	a2.10 m	a1.76 m
	b1.77 m	b1.40 m	b1.79 m	b1.83 dt	b1.70 dt	b1.63 dt
3	3.75 m	3.76 m	3.97 m	4.24 m	4.18 m	4.06 brs
4	a1.40 m	a1.41 m	a1.50 m	a1.68 m	a1.49 m	a1.55 dd
	b2.10 m	b2.10 m	b2.02 dd	b2.04 dd	b2.06 m	b1.37 td
5	–	–	–	–	–	1.53 m
6	3.14 d	3.04 d	3.42 brs	3.37 t	3.05 d	1.18 m
7	4.03 brs	4.07 brd	a1.73 dt	a1.57 m	a1.55 m	a0.90 td
	–	–	b1.56 m	b1.80 dt	b2.02 m	b1.86 dq
8	1.78 m	1.79 m	1.87 dt	1.89 m	1.86 dt	2.51 qd
9	1.45 dd	1.72 dd	2.12 m	2.16 brt	1.97 dd	0.89 dd
11	3.73 dd	5.00 dd	3.68 dd	3.77 dd	5.08 ddd	4.20 dq
12	4.70 d	3.35 dd	4.64 d	4.78 d	a1.20 dd	a1.69 dd
	–	–	–	–	b2.36 dd	b2.36 dd
14	1.70 dd	1.59 m	1.39 m	1.38 dt	1.44 dd	1.50 m
15	4.80 dt	4.80 dt	1.18 m	1.25 m	4.80 dt	a1.23 td
	–	–	1.67 m	1.69 m	–	b2.32 m
16	a1.81 dt	1.76 m	1.56 m	1.60 m	1.90 m	4.67 ddd
	b2.38 dt	2.36 dd	1.96 m	1.97 m	2.08 m	–
17	1.80 m	1.74 m	1.58 m	1.66 m	1.50 m	2.66 dd
18	0.86 s	0.79 s	0.82 s	0.82 s	0.76 s	–
19	1.18 s	1.02 s	1.27 s	1.23 s	1.04 s	1.09 s
20	1.20 m	1.18 m	1.16 m	1.19 m	0.96 m	–
21	0.91 d	1.10 d	0.91 d	0.92 d	0.96 brs	1.48 s
22	0.18 d	0.32 dt	0.22 dt	0.25 m	0.10 m	–
23	–	–	–	–	–	a1.80 t
	–	–	–	–	–	b2.13 dd
24	0.25 dq	0.26 dq	0.25 m	0.26 m	0.21 dq	2.29 m
25	1.55 m	1.56 m	1.56 m	1.60 m	1.56 m	–
26	0.86 d	0.86 d	0.82 d	0.86 d	0.84 d	2.30 s
27	0.93 d	0.93 d	0.95 d	0.98 d	0.93 d	0.99 s
28	0.91 d	0.92 d	0.94 d	0.95 d	0.87 d	0.96 d
29	0.91 s	0.90 s	0.91 s	0.91 s	0.89 s	
30	−0.11 dd	−0.11 dd	−0.09 dd	−0.07 dd	−0.11 dd	
	0.48 dd	0.48 dd	0.46 dd	0.47 dd	0.46 dd	
Ac	2.08 s	2.13 s	2.09 s	2.11 s	2.01 s	
	2.10 s	2.18 s			2.02 s	
7-OH	2.40 d	2.41 d				
11-OH						3.94 d
12-OH		2.21 d				

[a] Spectra taken in $CDCl_3$–CD_3OD (5:1).

The isomeric nature of compound **9** to **8** was inferred from its molecular formula $C_{34}H_{54}O_7$ ($\Delta=-0.8$ mmu for $[M-2H_2O]^+$) together with the presence of the same functional groups: a double bond (δ 5.60 dd; δ 145.8 s, 122.7 d) and two acetoxy groups (δ 2.05 s, 2.03 s; δ 173.1 s, 170.0 s, 21.8 q, 21.2 q; 1727 cm^{-1}). Two proton signals (δ 3.85 dd, 4.82 d) of **8** was replaced by the signals of δ 5.19 dd and 3.48 dd in **9**. By tracking COSY cross peaks [H-9(δ 1.95 dd)/H-11(δ 5.19 dd), H-11/H-12(δ 3.48 dd), and H-12/12-OH(δ 2.24 d)], an acetoxy group was placed at C-11 and a hydroxyl group at C-12. Thus **9** is gorgost-5-ene-3β,7α,11α,12β,15α-pentol 11,15-diacetate.

The molecular formula $C_{34}H_{54}O_8$ ($\Delta=0.0$ mmu for $[M-2H_2O]^+$) of **10** exhibited the presence of an additional oxygen atom as compared with **8** and **9**. With the absence of olefinic signals, the oxygen atom could be ascribed to an oxirane (δ 3.14 d; δ 63.7 s, 63.5 d). When the ^{13}C NMR data were compared with those of **7** and **8**, the C-1–C-10 portion was similar to that of **7** and the C-11–C-17 moiety to that of **8**. In addition, the presence of an acetoxy group at C-15α was confirmed by 2D NMR analysis. Combining these data the structure of **10** was elucidated as 5β,6β-epoxygorgostane-3β,7α,11α,12β,15α-pentol 12,15-diacetate.

Compound **11**, $C_{34}H_{54}O_8$ ($\Delta=-2.5$ mmu for $[M-2H_2O]^+$), was found to be an isomer of **10**, and the difference was clearly indicated by the ^{1}H NMR signals for H-11 (δ 5.00 dd) and H-12 (δ 3.35 dd) as observed in the relationship between **8** and **9**. Therefore, the structure was concluded as 5β,6β-epoxygorgostane-3β,7α,11α,12β,15α-pentol 11,15-diacetate (Tables 3 and 4).

Compound **12**, $C_{32}H_{54}O_6$ ($\Delta=+2.5$ mmu for $[M-H_2O-AcOH]^+$), was shown to have an acetoxy (δ 2.09 s, 4.64 d; δ 21.5 q, 85.8 d, 172.8 s), four hydroxyl groups (δ 3.42 brs, 3.68 dd, 3.97 m; δ 66.8 d, 72.6 d, 75.1 d, 76.2 s), and a cyclopropyl side chain in the same gorgostane skeleton as above. The absence of a double bond or an oxirane in **12** suggested the presence of 5,6-diol. The stereochemistry of the diol moiety (5α-OH, 6β-OH) was revealed by comparison of NMR data with those published[19] and also by observation of a small coupling for H-6 (δ 3.42 brs) with H-7αβ. Upfield shift of the C-3 signal (δ 66.8) of **12** relative

Table 4. ^{13}C NMR data for compounds **10**–**15** in $CDCl_3$

C#	10	11	12[a]	13[a]	14	15
1	38.0 t	37.6 t	33.7 t	74.6 d	74.2 d	31.5 t
2	30.9 t	30.8 t	30.5 t	36.5 t	37.9 t	28.6 t
3	69.1 d	68.8 d	66.8 d	63.0 d	63.8 d	66.4 d
4	42.0 t	42.0 t	40.2 t	40.3 t	42.2 t	35.1 t
5	63.7 s	63.4 s	76.2 s	77.7 s	61.9 s	39.7 d
6	63.5 d	63.4 d	75.1 d	73.9 d	63.2 d	27.5 t
7	66.9 d	66.9 d	33.6 t	34.1 t	31.2 t	32.4 t
8	32.5 d	32.1 d	28.6 d	28.8 d	27.1 d	29.7 d
9	47.9 d	44.9 d	50.5 d	45.8 d	44.9 d	57.8 d
10	35.3 s	35.2 s	39.8 s	41.9 s	40.6 s	36.4 s
11	73.5 d	77.3 d	72.6 d	71.2 d	72.4 d	66.8 d
12	84.0 d	82.3 d	85.8 d	86.5 d	45.5 t	40.8 t
13	46.8 s	48.0 s	47.0 s	46.9 s	43.4 s	53.0 s
14	52.0 d	51.3 d	53.1 d	52.8 d	58.3 d	56.8 d
15	76.2 d	76.5 d	23.7 t	23.7 t	75.2 d	34.9 t
16	37.1 t	37.4 t	27.5 t	27.2 t	38.4 t	80.2 d
17	55.4 d	55.9 d	57.4 d	57.0 d	55.3 d	59.9 d
18	11.1 q	10.2 q	9.9 q	9.9 q	13.4 q	182.5 s
19	15.7 q	16.3 q	16.3 q	15.6 q	16.0 q	13.8 q
20	32.0 d	32.6 d	33.8 d	32.7 d	34.6 d	90.1 s
21	22.3 q	22.3 q	22.0 q	21.8 q	20.3 q	18.8 q
22	29.8 d	29.9 d	30.2 d	29.7 d	31.8 d	116.7 s
23	25.3 s	25.3 s	25.1 s	24.8 s	25.8 s	38.5 t
24	50.5 d	50.5 d	50.1 d	50.4 d	50.6 d	41.1 d
25	32.5 d	32.5 d	31.9 d	31.9 d	31.7 d	85.6 s
26	21.2 q	21.9 q	21.5 q	21.4 q	21.3 q	29.0 q
27	22.2 q	22.3 q	22.0 q	22.0 q	22.1 q	23.0 q
28	15.2 q	15.0 q	15.1 q	15.1 q	15.2 q	13.9 q
29	13.9 q	13.8 q	13.6 q	13.4 q	14.3 q	–
30	21.2 t	21.5 t	21.0 t	20.8 t	21.2 t	–
Ac	21.6 q	21.4 q	21.5 q	21.8 q	21.4 q	–
	21.6 q	21.9 q			21.8 q	–
	169.8 s	169.8 s	172.8 s	172.8 s	169.5 s	
	172.9 s	172.9 s			170.6 s	

[a] Spectra taken in $CDCl_3$–CD_3OD (5:1).

to those of **5** (δ 69.3), **8** (δ 71.0), and **10** (δ 69.1) is due to 1,3-diaxial interaction between H-3α and 5α-OH. Thus, the structure was deduced as gorgostane-3β,5α,6β,11α,12β-pentol 12-acetate.

The molecular formula of compound **13**, $C_{32}H_{54}O_7$ ($\Delta=-4.2$ mmu), indicated the presence of an additional oxygen atom on that of **12**. The seven oxygen atoms were accounted for an acetoxy (δ 2.11; δ 86.5 d, 172.8 s) and five hydroxyl groups (δ 63.0 d, 71.2 d, 73.9 d, 74.6 d, 77.7 s). Overall features of the NMR spectra were similar to those of **12**. Thus, it was suggested to be a compound having an additional hydroxyl group as compared to **12**. The hydroxyl group was placed at 1α by COSY (H-1/H-2αβ, H-1/1-OH), small coupling between the oxymethine proton H-1 (δ 4.04 brs) and H-2αβ, and NOE between H-1 and H-19. The remaining portion of the molecule was the same as **12**. The structure of compound **13** is deduced as gorgostane-1α,3β,5α,6β,11α,12β-hexol 12-acetate.

Compound **14**, $C_{34}H_{54}O_7$ ($\Delta=-0.6$ mmu), was found to have two acetoxy (δ 2.01 s, 2.02 s, 4.80 dt, 5.08 ddd; 169.5 s, 170.6 s), two hydroxyl (δ 3.96 dd, 4.18 m; δ 74.2 d, 63.8 d), and an oxirane group (δ 3.05 d; δ 61.9 s, 63.2 d). The ^{13}C NMR signals for rings A and B portions were nearly identical with those of **6**, while the proton (δ 5.08 ddd, $J=5.2$, 11.4, 11.8 Hz) at acetoxy-bearing carbon (C-11) showed two large and one medium J values indicating the presence of two hydrogen atoms on C-12. Connectivity by 2D NMR analysis revealed the structure of **14** as 5β,6β-epoxygorgostane-1α,3β,11α,15α-tetrol 11,15-diacetate.

Compound **15**, $C_{28}H_{42}O_4$ ($\Delta=+0.7$ mmu), was shown to be a member of hippuristanols by the presence of a ketal signal (δ 116.7 s) and by the overall pattern of the NMR spectra. The spectral data indicated the presence of a lactone carbonyl (δ 182.5 s; 1746 cm^{-1}) and a hydroxyl group (δ 4.06 brs, 4.20 dq; δ 66.4 d, 66.8 d; 3490 cm^{-1}). Examination of the 2D NMR data allowed us to elucidate the structure of **15** as deacetyl derivative of the known steroid **16**.[6] As observed with **16**, a hydrogen bonded proton (δ 3.94 d, 11-OH) was also seen in **15**. On acetylation **15** gave a product identical with **16**, confirming that both compounds have the same absolute configurations. In order to determine the configuration of C-22, which was remained unsolved in **16**, a crystal of **15** was subjected to X-ray diffraction study. The result (Fig. 2) demonstrated it to be *S* and also confirmed the structure of **15** as deduced from spectral data.

2.2. Biological activity[20]

Except for the compounds **6** and **17**, the polyoxygenated steroids obtained in this study were submitted for screening of anti-MDR activity against drug resistant cancer cell lines. The results are summarized in Table 5. Most steroids showed moderate activity against drug-resistant cells (KB C2) expressing P-gp but not against cells (KB CV60) expressing multidrug resistance protein-1 (MRP1). Compounds **8** and **9** were most potent against KB C2.

3. Experimental

3.1. General

Optical rotation was recorded on a Jasco DIP-1000 polarimeter and IR spectra on a Jasco FTIR-300

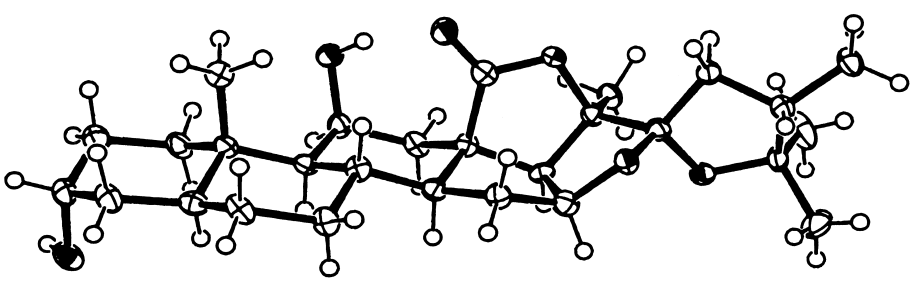


Figure 2. Computer-generated ORTEP drawing of compound **15**.

Table 5. Reversal of multidrug resistance

Compound	Dose (μg/mL)	Cell lines		
		KB 3-1	KB C2	KB CV60
4	10	35 ± 6.2	70 ± 6.5	54 ± 5.8
	3	–	68 ± 6.0	26 ± 5.8
5	30	9.3 ± 0.4	78.4 ± 1.7	27.2 ± 14.1
7	10	23 ± 4.8	79 ± 1.3	37 ± 3.9
	3	–	70 ± 2.2	16 ± 5.2
8	30	68.3 ± 1.8	87.5 ± 1.2	51.0 ± 2.1
	10	57.2 ± 3.7	79.1 ± 3.3	57.6 ± 1.6
	3	23.9 ± 4.8	87.8 ± 1.1	35.7 ± 3.7
	1	10.5 ± 6.8	61.9 ± 2.5	20.1 ± 5.3
9	30	97.4 ± 0.1	96.3 ± 0.3	96.8 ± 0.1
	10	87.3 ± 2.5	92.3 ± 1.6	52.3 ± 2.2
	3	26.4 ± 0.9	89.2 ± 1.2	41.0 ± 2.6
	1	6.2 ± 2.5	64.5 ± 1.4	16.0 ± 5.1
10	30	71.0 ± 1.2	90.7 ± 0.2	32.2 ± 3.7
	10	43.5 ± 4.1	77.8 ± 2.4	37.8 ± 4.1
	3	20.6 ± 9.0	87.4 ± 1.7	18.9 ± 5.7
	1	13.6 ± 2.9	47.8 ± 2.7	5.0 ± 4.4
11	30	97.6 ± 0.1	96.1 ± 0.6	96.9 ± 0.1
	10	42.1 ± 4.4	80.5 ± 1.4	52.4 ± 8.4
	3	20.6 ± 9.0	82.1 ± 2.5	16.6 ± 9.8
	1	–	25.3 ± 8.4	–
12	30	88.8 ± 0.8	92.4 ± 1.1	91.2 ± 1.7
	10	8.8 ± 2.3	35.1 ± 3.5	3.9 ± 10.4
13	30	9.6 ± 2.0	79.0 ± 1.9	39.6 ± 6.9
	10	6.0 ± 2.1	13.8 ± 1.8	9.6 ± 4.6
14	30	96.0 ± 0.5	95.1 ± 0.3	91.1 ± 0.7
	10	12.9 ± 5.9	88.8 ± 0.1	42.9 ± 5.8
15	30	52.6 ± 5.8	84.3 ± 0.9	36.0 ± 8.2
	10	26.1 ± 3.3	87.7 ± 0.4	36.2 ± 5.2
	3	0.4 ± 10.6	37.3 ± 4.5	14.2 ± 8.0
	1	5.2 ± 4.0	19.7 ± 12.0	1.8 ± 21.3

Percent inhibition of control ± SD.

spectrophotometer. ^{1}H and ^{13}C NMR spectra were taken on a Jeol A-500 NMR spectrometer. Mass spectra were measured either on a Jeol JMS-700, Hitachi M-2500, or VG 70SE instrument.

3.2. Animal material

A specimen of the gorgonian *I. hippuris* was collected at a reef on the island of Okinawa in August, 1988. Another sample of the gorgonian was collected at a reef slope (−10 to −30 m) in Yonaguni Island in January, 2000. The specimens were kept frozen until extraction. Voucher specimens (AT-5) are deposited at Department of Chemistry, Biology, and Marine Science, University of the Ryukyus.

3.3. Extraction and isolation

The Yonaguni specimen (5.0 kg, wet weight) was extracted by steeping in acetone for one day. The extraction was repeated three times. After concentration under vacuum, resulting aqueous suspension was extracted with EtOAc to give 55 g of a crude oil. The oil was passed on polystyrene gel by eluting first with MeOH and then with EtOAc. The MeOH eluate (32.5 g) was separated by vacuum flash chromatography on silica gel to give five fractions. The third fraction (1.54 g) was subjected to repeated separation on ODS HPLC (MeOH–MeCN–H_2O, 6:1:1) to give compounds **5** (2.8 mg), **6** (3.0 mg), **14** (1.5 mg), and **15** (19.8 mg). Similarly, the fourth fraction (2.31 g) yielded compounds **8**–**11** in the amounts of 7.7, 7.8, 25.2, and 6.1 mg, respectively. The fifth fraction (2.42 g) resulted in compounds **12** (5.9 mg) and **13** (3.3 mg).

The Okinawa specimen (10 kg, wet), after similar extraction and separation procedure as above, gave compounds **4** and **7** in yields of 151.7 and 81.3 mg, respectively, together with those previously reported.[7]

3.3.1. Gorgost-5-ene-3β,7α,11α,12β-tetrol 12-acetate (4). $[\alpha]_D = -12°$ ($CHCl_3$, *c* 0.2); mp 197–200°C; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2.

3.3.2. 5β,6β-Epoxygorgostane-3β,11α,12β-triol 12-acetate (5). Glass; $[\alpha]_D = -8.0°$ ($CHCl_3$, *c* 0.23); IR (neat) 3400, 1720, 1250 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; EIMS *m/z* 456 ($[M-AcOH]^+$), 152 (100 rel%); HREIMS *m/z* 456.3579 (calcd for $C_{30}H_{48}O_3$ 456.3600).

3.3.3. 5β,6β-Epoxygorgostane-1α,3β,11α,12β-tetrol 12-acetate (6). White amorphous solid; $[\alpha]_D = -12.8°$ ($CHCl_3$, *c* 0.25); IR (neat) 3417, 1714, 1246 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; EIMS *m/z* 472 ($[M-AcOH]^+$, 152 (100 rel%); HREIMS *m/z* 472.3515 (calcd for $C_{30}H_{48}O_3$ 472.3549).

3.3.4. 5β,6β-Epoxygorgostane-3β,7α,11α,12β-tetrol 12-acetate (7). White solid, $[\alpha]_D^{22} = +2.6°$ ($CHCl_3$, *c* 0.1); ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; EIMS *m/z* 514 ($[M-H_2O]^+$, 3), 496 (17), 472 (14), 152 (100 rel%); HREIMS *m/z* 514.3656 (calcd for $C_{32}H_{50}O_5$ 514.3655), 496.3555 (calcd for $C_{32}H_{48}O_4$ 496.3553).

3.3.5. Gorgost-5-ene-3β,7α,11α,12β,16α-pentol 12,16-diacetate (8). Glass; $[\alpha]_D^{25} = -2.4°$ ($CHCl_3$, *c* 0.64); IR (neat) 3417, 1729, 1255 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; EIMS *m/z* 538 ($[M-2H_2O]^+$, 5), 478 (4), 418 (14), 83 (100 rel%); FABMS *m/z* 557 ($[M-H_2O+H]^+$); HREIMS *m/z* 538.3660 (calcd for $C_{34}H_{50}O_5$ 538.3658); HRFABMS *m/z* 557.3799 (calcd for $C_{34}H_{53}O_6$ 557.3842).

3.3.6. Gorgost-5-ene-3β,7α,11α,12β,15α-pentol 11,15-diacetate (9). Glass; $[\alpha]_D^{25} = -9.2°$ ($CHCl_3$, *c* 0.45); IR (neat) 3442, 1727, 1371, 1241 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2; EIMS *m/z* 538 ($[M-2H_2O]^+$, 5), 478 (4), 418 (15), 83 (100 rel%); FABMS *m/z* 573 ($[M-H]^-$); HREIMS *m/z* 538.3647 (calcd for $C_{34}H_{50}O_5$ 538.3655).

3.3.7. 5β,6β-Epoxygorgostane-3β,7α,11α,12β,15α-pentol 11,15-diacetate (10). Glass; $[\alpha]_D^{25} = +24°$ ($CHCl_3$, *c* 0.62); IR (neat) 3444, 1731, 1241 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 3 and 4; EIMS *m/z* 554 ($[M-2H_2O]^+$, 7), 470 (10), 83 (100 rel%); FABMS *m/z* 591 ($[M+H]^+$); HREIMS *m/z* 554.3607 (calcd for $C_{34}H_{50}O_6$, 554.3607).

3.3.8. 5β,6β-Epoxygorgostane-3β,7α,11α,12β,16α-pentol 11,16-diacetate (11). Glass; $[\alpha]_D^{25} = +23°$ ($CHCl_3$, *c* 0.26); IR (neat) 3492, 3397, 1727, 1241 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 3 and 4; EIMS *m/z* 572 ($[M-H_2O]^+$, 14), 554 (15), 530 (31), 512 (70), 470 (56), 152 (100 rel%); ESIMS *m/z* 613 ($[M+Na]^+$), 591

($[M+H]^+$); FABMS *m/z* 591 ($[M+H]^+$), 573; HREIMS *m/z* 554.3582 (calcd for $C_{34}H_{50}O_6$, 554.3607); HRESIMS *m/z* 591.3934 (calcd for $C_{34}H_{55}O_8$ 591.3897).

3.3.9. Gorgostane-3β,5α,6β,11α,12β-pentol 12-acetate (12). Glass; $[\alpha]_D^{25}=-14°$ ($CHCl_3$, *c* 0.49); IR (neat) 3380, 1718, 1249 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$–CD_3OD): Tables 3 and 4; EIMS *m/z* 516 ($[M-H_2O]^+$, 1), 474 (9), 456 (10), 438 (12), 152 (100 rel%); HREIMS *m/z* 456.3626 (calcd for $C_{30}H_{48}O_3$ 456.3601).

3.3.10. Gorgostane-1α,3β,5α,6β,11α,12β-hexol 12-acetate (13). Glass; $[\alpha]_D^{25}=+5.0°$ ($CHCl_3$, *c* 0.49); IR (neat) 3392, 1718, 1247 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$–CD_3OD): Tables 3 and 4; FABMS *m/z* 551 ($[M+H]^+$), 473, 455; HRFABMS *m/z* 551.3906 (calcd for $C_{32}H_{55}O_7$ 551.3948).

3.3.11. 5β,6β-Epoxygorgostane-1α,3β,11α,15α-tetrol 11,15-diacetate (14). Glass; $[\alpha]_D^{25}=+32°$ ($CHCl_3$, *c* 0.04); IR (neat) 3446, 1731, 1241 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): Tables 3 and 4; EIMS *m/z* 556 ($[M-H_2O]^+$, 1), 496 (19), 436 (35), 69 (100 rel%); ESIMS *m/z* 597 ($[M+Na]^+$), 575 ($[M+H]^+$); FABMS *m/z* 575 ($[M+H]^+$), 557 ($[M-H_2O+H]^+$); HRESIMS *m/z* 597.3761 (calcd for $C_{34}H_{54}O_7Na$ 597.3767).

3.3.12. Compound 15. Colorless plates; mp 225–226°C (MeOH–H_2O); $[\alpha]_D=-22°$ ($CHCl_3$, *c* 0.85); IR (neat) 3490, 1746, 1277 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): Tables 3 and 4; EIMS *m/z* 474 (M^+), 473, 239 (100 rel%); FABMS *m/z* 475 ($[M+H]^+$), 457, 439; HREIMS *m/z* 474.2988 (calcd for $C_{28}H_{42}O_6$ 474.2981).

3.3.13. Oxidation of 4 to give 17. A solution of **4** (4.0 mg) and MCPBA (10 mg) in CH_2Cl_2 (1.0 mL) was allowed to stand at 0°C for 18 h. The solution was washed with sodium bicarbonate solution, and the organic layer was concentrated. The residue was separated on preparative TLC (silica, hexane–EtOAc, 3:10) to give **17** (3.0 mg, 73%) as a white solid. Compound **17**: $[\alpha]_D^{22}=-36°$ ($CHCl_3$, *c* 0.3); ^1H NMR ($CDCl_3$) δ 4.67 (1H, d, *J*=9 Hz), 3.88 (2H, m), 3.70 (1H, q, *J*=9 Hz), 3.49 (1H, s), 3.25 (1H, d, *J*=5 Hz), 2.10 (3H, s), 0.94 (3H, d, *J*=7 Hz), 0.92 (3H, d, *J*=7 Hz), 0.91 (3H, d, *J*=7 Hz), 0.90 (3H, s), 0.85 (3H, d, *J*=7 Hz), 0.76 (3H, s), 0.46 (1H, m), 0.23 (2H, m), −0.11 (1H, m); ^{13}C NMR ($CDCl_3$) δ 173.0 s, 85.8 d, 72.9 d, 69.4 s, 68.5 d, 64.2 d, 62.8 d, 57.1 d, 50.6 d, 46.8 d, 46.2 s, 44.3 d, 39.5 t, 37.1 s, 36.8 d, 34.1 t, 33.3 d, 32.1 d, 31.1 t, 30.2 d, 27.7 t, 25.3 s, 23.9 t, 22.4 q, 22.2 q, 21.7 q, 21.4 q, 21.3 t, 16.5 q, 15.4 q, 13.8 q, 10.0 q; EIMS *m/z* 514 ($[M-H_2O]^+$, 2), 472 (10), 152 (100 rel%); HREIMS *m/z* 472.3520 (calcd for $C_{30}H_{48}O_4$ 472.3549).

3.4. Acetylation of 15 to yield 16

A mixture of **15** (3.1 mg), acetic anhydride (0.2 mL), and pyridine (0.2 mL) was kept standing at room temperature for 10 h. Then, the mixture was partitioned between Et_2O and water, and the organic layer was dried over Na_2SO_4, and concentrated. After crystallization from acetone, **16** (76%) was obtained. The sample showed identical R_f value on TLC, ^1H NMR, and IR spectra with those of an authentic sample.[6]

3.5. X-Ray diffraction of 4

Colorless block crystals of **4** were obtained by recrystallization (MeOH–H_2O). The crystal (0.3×0.3×0.7 mm) belongs to the orthorhombic system, space group $P2_12_12_1$ with $a=7.9609(5)$ Å, $b=10.5111(9)$ Å, $c=35.678(2)$ Å, $V=2985.4(3)$ Å^3, $Z=4$, $D_{calcd}=1.145$ g/cm^3, λ(Mo Kα)=0.71069 Å. Intensity data were measured on a Rigaku RAXIS-RAPID diffractometer up to 2θ of 55°. A total of 3893 reflections were collected. The structure was solved by direct method (SIR 92) and refined by a full-matrix least squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to the final $R=0.072$, $R_w=0.084$ for 3011 observed reflections [$I>3.00\sigma(I)$] and 386 variable parameters. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 182359. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

3.6. X-Ray diffraction of 15

Colorless plates of **15** were obtained by recrystallization (MeOH–H_2O). The crystal (0.12×0.23×0.38 mm) belongs to the orthorhombic system, space group $P2_12_12_1$ with $a=10.4991(3)$ Å, $b=33.5883(8)$ Å, $c=7.7578(2)$ Å, $V=2735.8(1)$ Å^3, $Z=4$, $D_{calcd}=1.152$ g/cm^3, λ(Mo Kα)=0.71069 Å. Intensity data were measured on a Rigaku RAXIS-RAPID diffractometer up to 2θ of 55°. A total of 3597 reflections were collected. The structure was solved by direct method (SIR 92) and refined by a full-matrix least squares procedure. The non-hydrogen atoms were given anisotropic thermal parameters. The refinement converged to the final $R=0.058$, $R_w=0.077$ for 2574 observed reflections [$I>3.00\sigma(I)$] and 325 variable parameters. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 182360. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

3.7. Anti-MDR screening[20]

The bioassay was carried out by Dr S. Aoki, Osaka University.

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