SYNTHESIS OF DIOLMYCIN ANALOGS AND THEIR ANTICOCCIDIAL ACTIVITIES

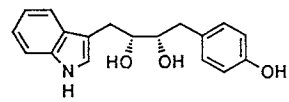
Sir:

In the course of our screening for anticoccidial antibiotics, diolmycins have been isolated from the fermentation broth of *Streptomyces* sp. WK-2955[1]. Diolmycins had four active compounds; A series were consisted of an indole unit and a phenol unit across butanediol, and B series were consisted of di-phenol units across butanediol. Diolmycins A1 (**1**) and A2 (**2**) were stereoisomers, and B1 (**3**) and B2 (**4**) were also stereoisomers. Diolmycins A1 (**1**) and B1 (**3**) were *erythro*-diols, however, diolmycins A2 (**2**) and B2 (**4**) were *threo*-diols (Fig. 1)[2].

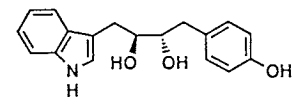
We have established the synthesis of racemic diolmycin A1 (**1**) *via* stereoselective Wittig reaction followed by dihydroxylation with osmium tetroxide to confirm the relative configuration of **1**. Furthermore, we have established the asymmetric synthesis of (−)-diolmycin A1 (**1**) *via* kinetic resolution of racemic allyl alcohol by enantioselective epoxidation according to the method of K. B. SHARPLESS[3] followed by coupling reaction between epoxide and indole with a Lewis acid[4] to determine the absolute configuration of natural diolmycin A1 (**1**)[5]. The order of the *in vitro* anticoccidial activity using monensin-resistant *Eimeria tenella* was diolmycin A1 (**1**) > A2 (**2**) ≫ B1 (**3**) = B2 (**4**), indicating that the indole unit was important for anticoccidial activity. In this communication, we report synthesis of the di-indole compound (**10**) to clarify the structure-activity relationships of diolmycins.

The synthesis of di-indole compound (**10**) was

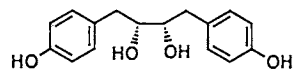
Fig. 1. Structures of diolmycins A1 (**1**), A2 (**2**), B1 (**3**) and B2 (**4**).



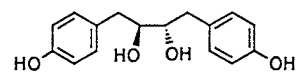
Diolmycin A1 (**1**)



Diolmycin A2 (**2**)*



Diolmycin B1 (**3**)



Diolmycin B2 (**4**)

* Relative configuration.

Scheme 1.

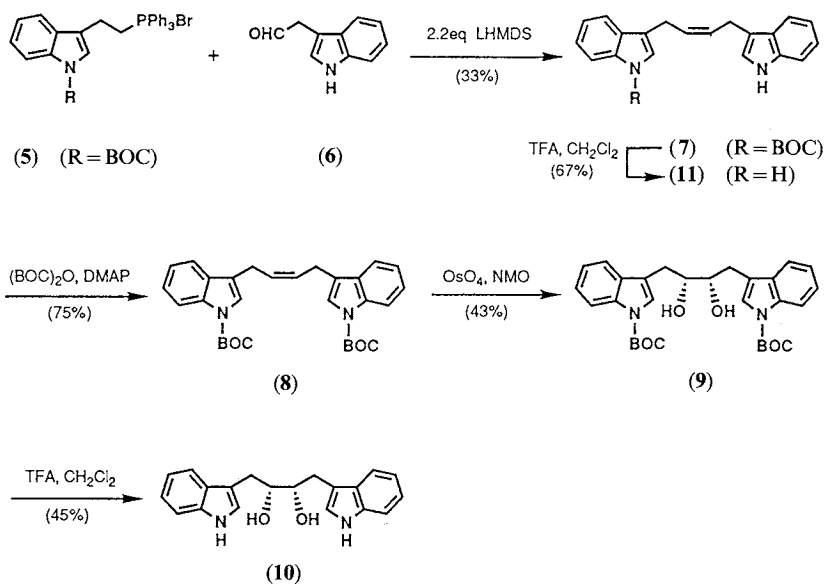


Table 1. Anticoccidial activity of diolmycin analogs in an *in vitro* assay.

Compound	A activity[a]	B CT[b]	B/A level[c]
(−)-Diolmycin A1 (**1**)	0.5	10	20
(+)-Diolmycin A1	1.0	2.0	2
(**7**)	0.5	10	20
(**8**)	10	50	5
(**9**)	—[d]	5.0	—
(**10**)	—[d]	0.05	—
(**11**)	0.5	10	20
(**2**)	5	50	10
(**3**)	50	>50	1
(**4**)	50	>50	1

BHK-21 cells stained with hematoxylin solution was microscopically observed. In control experiments (no drug) infected sporocysts grew in the cells to form mature shizonts.

[a] No mature shizonts observed in the cells when the drug was added to the culture medium at the indicated concentrations.

[b] No BHK-21 cells observed when the drug was added to the culture medium at the indicated concentrations.

[c] The B/A level was specificity for the activity. (A: anticoccidial activity, B: cytotoxicity).

[d] No anticoccidial activity.

accomplished *via* stereoselective Wittig reaction followed by osmium oxidation as outlined in scheme 1 in a similar manner to that described for the preparation of racemic diolmycin A1[2]. The Wittig reaction was carried out between phosphonium bromide (**5**)[2] and aldehyde (**6**). For the formation of the unstable yilid, **5** was added to 2.2 equivalents of lithium *bis*(trimethylsilyl)amide (LHMDS) at 0°C for 30 minutes. The resulting yilid quenched by **6** at 0°C gave the (*Z*)-olefin (**7**) predominantly[6] in 33% yield. The coupling constant between olefinic protons of the olefin (**7**) was 10.7 Hz by decoupling. To prevent oxidation of the indole, **7** was protected with *t*-butoxycarbonyl (BOC) by treatment with BOC anhydride and 4-dimethylaminopyridine (DMAP) in acetonitrile[7] in 75% yield to afford **8**. The protected (*Z*)-olefin (**8**) was oxidized by catalytic osmium tetroxide and 4-methylmorpholine *N*-oxide (NMO) in dioxane[8] to yield *erythro*-diol (**9**) in 43% yield. Finally, the *t*-butoxycarbonyl protecting group of indole was removed by treatment with trifluoroacetic acid (TFA) in dichloromethane[9] to obtain di-indole compound (**10**) in 45% yield. ^{1}H NMR (270 MHz, CD_3OD) δ 3.16~3.20 (4H, m), 3.80 (2H, d, J=4.6 Hz), 6.82~6.99 (6H, m), 7.22 (2H, d, J=4.0 Hz), 7.44 (2H, d, J=4.0 Hz); IR ($CHCl_3$) cm^{-1} 3700, 3490 and 1600; Mass *m/z* 320 (M^+), HREI-MS calcd for $C_{20}H_{20}N_2O_2$: 320.1496, found: 320.1524. **11** was also obtained by the same treatment of **7** in 67% yield.

Anticoccidial activity in an *in vitro* assay and cytotoxicity[10] of the synthetic compounds were summarized in Table 1. (+)-Diolmycin A1 which is an enantiomer of natural (−)-diolmycin A1 (**1**) showed an anticoccidial activity at concentrations ranging above 1.0 mg/ml, which was half as active as that of **1**, otherwise its cytotoxicity increased five times than **1**. The di-indole compound (**10**) showed the very strong cytotoxicity which was 200 times as strong as that of **1**. On the other hand, the olefin compounds **7** and **11** showed the same anticoccidial activity and cytotoxicity as **1**.

Compounds **7** and **11** are expected as a new lead for anticoccidial reagents.

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