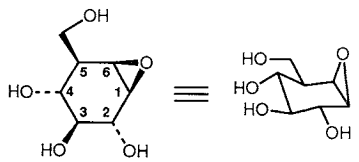
ENANTIOSPECIFIC SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,6-*epi*-CYCLOPHELLITOL

Sir:

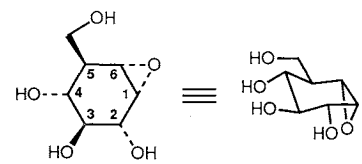
Cyclophellitol[1] (**1**) is a novel β-glucosidase inhibitor isolated from culture filtrates of mushroom, *Phellinus* sp. and, structurally, is the fully oxygenated cyclohexane, which corresponds to a carba analogue of D-glucopyranose. Recently, cyclophellitol (**1**) has been synthesized from L-glucose through the stereospecific intramolecular cyloaddition in our laboratories[2].

In order to provide additional insight into the mode of action of cyclophellitol, we now communicate the synthesis and glycosidase inhibiting activities of the unnatural epoxide diastereomer of **1**, 1,6-*epi*-cyclophellitol (**2**). The synthesis originated from methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (**3**)[3]. Oxidation (oxalyl chloride, DMSO, Et_3N, −78°C, 30 minutes) of **3** to give the aldehyde, followed by a Wittig reaction with $Ph_3P=CH_2$ in benzene provided the olefin **4**: $[\alpha]_D^{25}$ +30° (*c* 0.50, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 3.36 (3H, s, OCH_3), 4.73 (1H, d, $J_{1,2}=4.2$ Hz, 1-H), 5.15 (1H, ddd, $J=1.2$, 1.2 and 10.2 Hz, 7_{cis}-H), 5.29 (1H, ddd, $J=1.2$, 1.2 and 17.0 Hz, 7_{trans}-H), 5.84 (1H, ddd, $J=6.0$, 10.2 and 17.0 Hz, 6-H); *Anal* Calcd for $C_{29}H_{32}O_5$: C 75.36, H 7.00. Found: C 75.67, H 6.84. Acetolysis (Ac_2O, H_2SO_4, 0°C, 10 minutes) and de-*O*-acetylation (MeONa, MeOH, 25°C, 30 minutes) led to the corresponding hemiacetal, which was treated with $NH_2OH \cdot HCl$ in pyridine (25°C, 3 hours) to give the oxime **5** in 90% overall yield: $[\alpha]_D^{25}$ +51° (*c* 0.53, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 2.61 (0.3H, d, $J=9.0$ Hz, 5-OH (*Z*-oxime)), 2.66 (0.7H, d, $J=9.0$ Hz, 5-OH (*E*-oxime)), 5.23 (1H, ddd, $J=2.0$, 2.0 and 10.4 Hz, 7_{cis}-H), 5.40 (1H, ddd, $J=2.0$, 2.0 and 17.0 Hz, 7_{trans}-H), 6.03 (1H, ddd, $J=4.0$, 10.4 and 17.0 Hz, 6-H), 6.96 (0.3H, d, $J=6.2$ Hz, 1-H (*Z*-oxime)), 7.44 (0.7H, d, $J=8.0$ Hz, 1-H (*E*-oxime)); *Anal* Calcd for $C_{28}H_{31}NO_5$: C 72.87, H 6.77, N 3.04. Found: C 72.84, H 6.73, N 3.02. The oxime **5** was submitted to the intramolecular cycloaddition[2] by using 5% aq NaOCl in toluene at 100°C for 30 minutes to produce the intermediary nitrile oxide. Expectedly[2], two adducts **6** and **7** were isolated by silica gel column chromatography (benzene - ethyl acetate (4:1)): **6**: 15% yeild; mp 142~144°C (recrystallization from ethyl acetate - hexane); $[\alpha]_D^{25}$ +133° (*c* 0.50, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 2.14 (1H, d, $J=5.0$ Hz, 6-OH†), 3.77 (1H, m, 5-H), 3.99 (1H, dd, $J_{2,3}=9.2$ Hz, $J_{1,2}=2.0$ Hz, 2-H), 4.74 (1H, dd, $J_{3,5}=1.0$ Hz, 3-H); *Anal* Calcd for $C_{28}H_{29}NO_5$: C 73.19, H 6.36, N 3.05. Found: C 72.89, H 6.54, N 2.99. **7**: 75% yield; $[\alpha]_D^{25}$ −54° (*c* 0.50, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 2.57 (1H, d, $J=1.4$ Hz, 6-OH), 3.43 (1H, ddd, $J=10.0$, 10.0 and 10.0 Hz, 5-H), 3.78 (1H, dd, $J=2.2$ and 9.4 Hz); *Anal* Calcd for $C_{28}H_{29}NO_5$: C 73.19, H 6.36, N 3.05. Found: C 72.89, H 6.29, N 3.08.

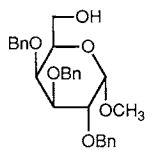
The desired isoxazoline **6** was converted by acidic hydrogenolysis(1 atm H_2, Raney Ni-W4, dioxane - aq AcOH) into the keto-alcohol **8** in 75% yield: $[\alpha]_D^{25}$ +88° (*c* 0.50, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 2.76 (1H, dd, $J=4.2$ and 10.2 Hz, CH_2OH), 2.89 (1H, m, 5-H), 4.03 (1H, dd,



Cyclophellitol (**1**)



1,6-*epi*-Cyclophellitol (**2**)



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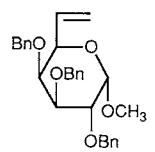

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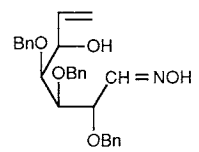

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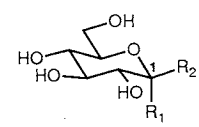
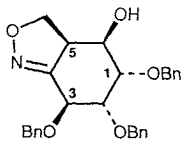
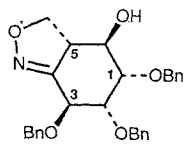


13α $R_1=OR$ $R_2=H$
13β $R_1=H$ $R_2=OR$

† The carbon-numbering protocol of **6**~**12** anticipates conveniently the construction of 1,6-*epi*-cyclophellitol (**2**).



6†



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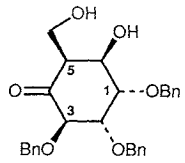

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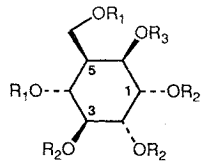

9 $R_1=R_3=H$ $R_2=Bn$
10 $R_1=$DEIPS $R_2=Bn$ $R_3=H$
11 $R_1=$DEIPS $R_2=Bn$ $R_3=Ms$
12 $R_1=R_2=H$ $R_3=Ms$

$J_{1,2}=J_{1,6}=3.8$ Hz, 1-H), 4.05 (1H, s, 6-OH), 4.13 (1H, dd, $J_{2,3}=10.0$ Hz, 2-H), 4.06 (1H, d, 3-H); *Anal* Calcd for $C_{28}H_{30}O_6$: C 72.71, H 6.54. Found: C 72.49, H 6.43. Also, the similar hydrogenolysis of the undesired isoxazoline **7** gave, through natural and reasonable epimerization at C-5 position by the keto-enol tautomerism, the desired keto-alcohol **8** as the major product in 70% yield. The ketone **8** was stereoselectively reduced by BH_3-Me_2S in THF at 0°C for 30 minutes to afford the alcohol **9** in 80% yield: $[\alpha]_D^{25}$ +11° (*c* 0.50, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.97 (1H, m, 5-H), 2.26, 2.53, and 3.30 (each 1H, each br, 3×OH); *Anal* Calcd for $C_{28}H_{32}O_4$: C 72.40, H 6.94. Found: C 72.64, H 6.84.

Compound **9** contains three kinds of hydroxyl groups, namely, the primary, C-4 equatorial, and C-6 axial hydroxyl groups, which are expected to be selectively protected. In fact, selective silylation of **9** with diethylisopropylsilyl chloride[4] and imidazole in DMF at 0°C for 4 hours gave the desired di-*O*-silylated compound **10** as the major product without protection of the axial hydroxyl group in 60% yield: MP 69~72°C (recrystallization from MeOH); $[\alpha]_D^{25}$ +34° (*c* 0.50, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.55~0.75 (8H, m, 2×Si(CH_2CH_3)$_2$), 0.85~1.07 (26H, m, 2×Si(CH_2CH_3)$_2$ and 2×Si^{i}Pr), 1.93 (1H, m, 5-H), 3.73 (1H, dd, $J_{2,3}=10.0$ Hz, $J_{3,4}=8.4$ Hz, 3-H), 3.85 (1H, dd, $J_{1,2}=J_{1,6}=3.2$ Hz, 1-H), 3.87 (1H, dd, $J=3.8$ and 10.2 Hz, one of CH_2OH), 3.95 (1H, dd, 2-H), 3.96 (1H, brs, 6-OH), 4.06 (1H, dd, $J_{4,5}=10.6$ Hz, 4-H), 4.17 (2H, dd, $J=3.8$ and 10.2 Hz, one of CH_2OH contaminated with 6-H); *Anal* Calcd for $C_{42}H_{64}O_6Si_2$: C 69.96, H 8.95. Found: C 70.05, H 8.68. The diethylisopropylsilyl protecting group was first developed by us[4] and found to be effectively removed by hydrogenolysis[5] as well as mild acidic conditions. Mesylation of **10** (MsCl, pyridine, 25°C, 12 hours) afforded **11**, which was subjected to the hydrogenolysis using 1 atm H_2 and $Pd(OH)_2$ in MeOH to generate the bare mesylate **12** in 90% overall yield: $[\alpha]_D^{25}$ +89° (*c* 1.00, H_2O); ^{1}H NMR (270 MHz, D_2O) δ 2.22 (1H, m, 5-H), 3.25 (3H, s, OMs), 3.39 (1H, dd, $J_{3,4}=9.8$ Hz, $J_{4,5}=11.0$ Hz, 4-H), 3.59 (1H, dd, $J_{2,3}=9.8$ Hz, 3-H), 3.61 (1H, dd, $J=11.0$ and 11.0 Hz, one of CH_2OH), 3.73 (1H, dd, $J_{1,2}=3.4$ Hz, 2-H), 3.98 (1H, dd, $J=4.2$ and 11.0 Hz, one of CH_2OH), 4.21 (1H, dd, $J_{1,6}=3.4$ Hz, 1-H), 4.98 (1H, dd, $J_{5,6}=$ 4.0 Hz, 6-H); *Anal* Calcd for $C_8H_{16}O_8S\cdot\frac{1}{2}H_2O$: C 34.17, H 6.09. Found: C 34.29, H 6.29.

Finally, treatment of **12** with MeONa in MeOH afforded the crystalline 1,6-*epi*-cyclophellitol (**2**) in 80% yield: MP 150~152°C (crystallization from MeOH); $[\alpha]_D^{25}$ +80° (*c* 0.36, H_2O); ^{1}H NMR (270 MHz, D_2O) δ 2.04 (1H, ddd, $J_{4,5}=8.8$ Hz, $J_{5,6}=0$ Hz, $J_{5,CH_2OH}=3.4$ and 5.8 Hz, 5-H), 3.33 (1H, d, $J_{1,6}=3.9$ Hz, 6-H), 3.34 (1H, dd, $J_{3,4}=$ 9.8 Hz, 4-H), 3.42 (1H, dd, $J_{2,3}=8.4$ Hz, 3-H), 3.46 (1H, dd, $J_{1,2}=2.3$ Hz, 1-H), 3.78 (1H, dd, $J_{gem}=$ 11.2 Hz, one of CH_2OH), 3.91 (1H, dd, 2-H), 3.91 (1H, dd, one of CH_2OH); *Anal* Calcd for $C_7H_{12}O_5$: C 47.73, H 6.87. Found: C 47.42, H 6.45.

The glycosidase inhibiting activities of **2** were generally assayed according to the method reported by SAUL *et al.*[1,6] In dramatic contrast to natural cyclophellitol (**1**) which inhibited only almond β-glucosidase activity for 50% at 0.8 μg/ml[1], the *epi*-epoxide **2** exhibited the inhibiting activity only against baker yeast α-glucosidase at IC_{50} 10 μg/ml. Structurally, cyclophellitol (**1**) has a *quasi*-equatorially oriented C1-O bond, which corresponds to an equatorial C1-O bond of β-glucopyranoside (**13**β), while the *epi*-cyclophellitol **2** has a *quasi*-axial C1-O bond corresponding to an axial C1-O bond of α-D-glucopyranoside (**13**α). The glucosidase inhibiting activities of **1** and **2** emphasized that the α- and β-glucosidases recognized especially their C-1 positions as those of α- and β-glucopyranosides.

† See footnote on p. 456.

Consequently, cyclophellitol (**1**) and its *epi*-epoxide, 1,6-*epi*-cyclophellitol (**2**) serve as the antagonists of β- and α-glucopyranosides, respectively.

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