SYNTHESES AND ENZYME INHIBITING ACTIVITIES OF CYCLOPHELLITOL ANALOGS

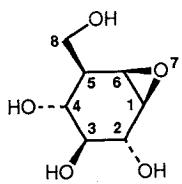
Sir:

Cyclophellitol[1] (**1**) was isolated from culture filtrates of mushroom, *Phellinus* sp., and exhibits a very high β-glucosidase inhibiting activity. Recently, we have synthesized[2,3] cyclophellitol (**1**) from L-glucose through the stereospecific intramolecular cycloaddition of a nitrile oxide to an olefin. In order to provide additional insight into the mode of action of cyclophellitol (**1**), we have also synthesized the unnatural epoxide diastereomer of **1**, 1,6-*epi*-cyclophellitol (**2**)[3,4], which proved to be an α-glucosidase inhibitor. As part of an ongoing program to clarify the mode of action of glucosidase inhibition, we attempted to make some analogs having different configurations and functionalities on the cyclohexane unit. We describe in this communication the syntheses and the glycosidase inhibiting activities of the α-manno type analog **3** and the aziridine analog **4** of cyclophellitol (**1**). The α-manno analog **3** was expected to inhibit the α-mannosidase activity[4] and the aziridine analog **4** was expected to show strong β-glucosidase inhibiting activity[5]. The α-manno analog **3** was derived from the isoxazoline **5** which was the key intermediate in 1,6-*epi*-cyclophellitol (**2**) synthesis[3,4]. The aziridine analog **4** was derived from 1,6-*epi*-cyclophellitol (**2**) itself. Although there are many polyhydroxylated nitrogen-containing heterocycles (*e.g.* nojirimycin[6], swainsonine[7], and their analogs[5]), the compound **4** is, to our knowledge, the first 7-azabicyclo[4.1.0]-heptane derivative having very high glucosidase inhibiting activity (*vide infra*).

The synthesis of **3** began with the isoxazoline **5**[3,4]. In contrast to the synthesis of **2**[3,4], it was necessary to open the isoxazoline without epimerization at C-5 position†. Therefore, the hydrogenolysis of **5** was conducted under the conditions of 1 atm of H_2 and Raney Ni-W4 in dioxane and in the presence of $B(OH)_3$[8], which was known to inhibit the epimerization of the center adjacent to the carbonyl function, to afford the keto-alcohol **6**[3,4] in a quantitative yield as a single product. Reduction of **6** with $Zn(BH_4)_2$ in THF in the presence of $MgBr_2$ at 0°C for 1 hour gave the alcohol **7** (60%) and its C-6 epimer (17%) which were easily separated by silica gel column chromatography (benzene-ethyl acetate (2:3)): **7**: $[\alpha]_D^{25}$ +12° (*c* 0.50, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 3.70 (1H, dd, $J_{2,3}=3.2$ Hz, $J_{3,4}=9.4$ Hz, 3-H), 4.30 (1H, ddd, $J_{4,5}=10.4$ Hz, $J_{4,OH}=2.0$ Hz, 4-H) (the corresponding triacetate: δ 1.92, 2.01, 2.02 (each 3H, each s, 3 × OAc), 5.11 (1H, dd, $J_{5,6}=J_{1,6}=4.2$ Hz, 6-H), 5.48 (1H, dd, $J_{3,4}=J_{4,5}=8.0$ Hz, 4-H)); *Anal* Calcd for $C_{28}H_{32}O_6$: C 72.40, H 6.94. Found: C 72.21, H 6.94. The stereochemistry was confirmed by the ^1H NMR analyses of compounds **7**, **8**, **9**, and **3**. Regioselective benzylidenation of **7** ($PhCH(OMe)_2$, camphorsulfonic acid, DMF, 50°C, 1 hour) followed by mesylation (MsCl, pyridine, 25°C, 12 hours) afforded **8** ($[\alpha]_D^{25}$ +9.3° (*c* 0.30, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 2.78 (3H, s, OMs), 4.75 (1H, dd, $J_{1,6}=J_{5,6}=2.2$ Hz, 6-H), 5.63 (1H, s, OC*H*(Ph)O)), which was subjected to the hydrogenolysis using 1 atm of H_2 and $Pd(OH)_2$ in MeOH to generate the mesylate **9** in 50% overall yield from **7**: $[\alpha]_D^{25}$ +15° (*c* 0.35, H_2O); ^1H NMR (270 MHz, D_2O, DOH=4.80) δ 3.26 (3H, s, OMs), 3.84 (1H, dd, $J_{2,3}=3.6$ Hz, $J_{3,4}=7.8$ Hz, 3-H), 3.89 (1H, dd, $J_{3,4}=J_{4,5}=7.8$ Hz, 4-H), 4.18 (1H, dd, $J_{1,2}=J_{1,6}=5.0$ Hz, 1-H), 4.93 (1H, dd, $J_{5,6}=5.0$ Hz, 6-H).

Finally, treatment of **9** with MeONa in MeOH (0°C, 3 hours) afforded the α-manno type compound **3** in 60% yield: $[\alpha]_D^{25}$ −76° (*c* 0.10, H_2O): ^1H NMR (270 MHz, CD_3OD) δ 1.93 (1H, m, 5-H), 3.17 (1H, d, $J_{1,6}=3.0$ Hz, $J_{5,6}=0$ Hz, 6-H), 3.22 (1H, dd, $J_{1,2}=3.0$ Hz, 1-H), 3.41 (1H, dd, $J_{2,3}=3.0$ Hz, $J_{3,4}=9.8$ Hz, 3-H), 3.45 (1H, dd, $J_{4,5}=9.8$ Hz, 4-H), 3.66 (1H, dd, $J_{5,8}=7.8$ Hz, $J_{gem}=10.6$ Hz, 8-H), 3.77 (1H, dd, $J_{5,8'}=4.0$ Hz, 8'-H), 4.27 (1H, dd, 2-H).

The aziridine type compound **4** was prepared as



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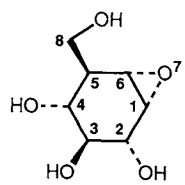

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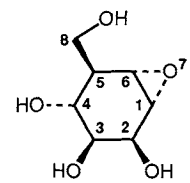

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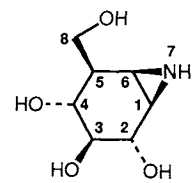
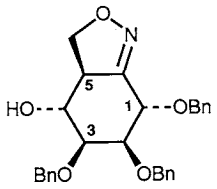


4

† The carbon-numbering protocol of **5**~**13** anticiptates conveniently the construction of **3** and **4**.



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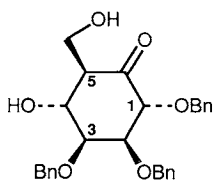

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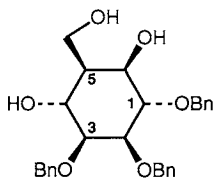

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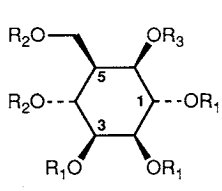
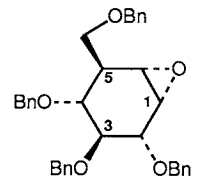


8 R_1 = Bn, R_2 = benzylidene, R_3 = Ms
9 $R_1 = R_2$ = H, R_3 = Ms



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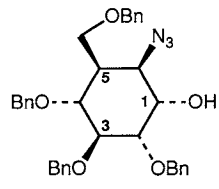

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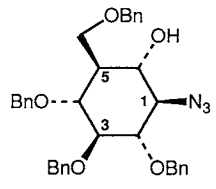

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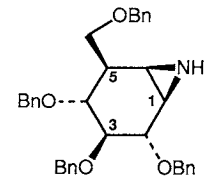

13

follows. 1,6-*epi*-Cyclophellitol (**2**) was benzylated with BnBr and NaH in DMF at 25°C for 0.5 hour to give the tetra-*O*-benzyl derivative **10** in 95% yield: $[\alpha]_D^{25}$ +60° (*c* 0.50, $CHCl_3$); mp 103~104°C (recrystallization from ethyl acetate-hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.21 (1H, ddd, $J_{4,5}$=9.6 Hz, $J_{5,6}$=0 Hz, $J_{5,8}$=$J_{5,8'}$=3.6 Hz, 5-H), 3.16 (1H, d, $J_{1,6}$=4.0 Hz, 6-H), 3.32 (1H, dd, $J_{1,2}$=2.0 Hz, 1-H); *Anal* Calcd for $C_{35}H_{36}O_5$: C 78.34, H 6.76. Found: C 78.76, H 6.81. The epoxide-ring opening of **10** with NaN_3 in DMF at 110°C for 12 hours afforded a mixture of **11** and **12** which was separated by silica gel column chromatography (chloroform-ethyl acetate (20:1)): **11**: 50% yield: $[\alpha]_D^{25}$ +32° (*c* 0.44, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.53 (1H, br s, 1-OH), 4.07 (1H, br dd, $J_{1,2}$=$J_{1,6}$=3.0 Hz, 1-H), 4.17 (1H, dd, $J_{5,6}$=3.0 Hz, 6-H); *Anal* Calcd for $C_{35}H_{37}N_3O_5$: C 72.52, H 6.43, N 7.25. Found: C 72.80, H 6.20, N 7.12. **12**: 25% yield: $[\alpha]_D^{25}$ +61° (*c* 0.41, $CHCl_3$); MP 137~139°C (recrystallization from ethyl acetate-hexane); ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.97 (1H, d, $J_{OH,6}$=2.0 Hz, 6-OH); *Anal* Calcd for $C_{35}H_{37}N_3O_5$: C 72.52, H 6.43, N 7.25. Found: C 72.36, H 6.25, N 7.28. Without separation, the mixture of **11** and **12** was subjected to reduction with PPh_3 in toluene (110°C, 0.5 hour)[9,10] to afford a single aziridine **13** in 60% yield, because both compounds **11** and **12** gave the desired aziridine **13**: $[\alpha]_D^{25}$ +94° (*c* 0.35, $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.35 (1H, dd, $J_{1,2}$=0 Hz, $J_{1,6}$=6.0 Hz, 1-H), 2.63 (1H, dd, $J_{5,6}$=3.0 Hz, 6-H); *Anal* Calcd for $C_{35}H_{37}NO_4$: C 78.48, H 6.96, N 2.62. Found: C 78.14, H 6.77, N 2.56.

Finally, de-*O*-benzylation of **13** (Li, NH_3, ether, −78°C, 1 hour) afforded the desired aziridine analog **4** in 60% yield: $[\alpha]_D^{25}$ +104° (*c* 0.10, H_2O); ^{1}H NMR (270 MHz, D_2O, DOH=4.80) δ 2.06 (1H, m, 5-H), 2.34 (1H, d, $J_{1,2}$=0 Hz, $J_{1,6}$=6.0 Hz, 1-H), 2.62 (1H, dd, $J_{5,6}$=4.0 Hz, 6-H), 3.07 (1H, dd, $J_{3,4}$=$J_{4,5}$=10.0 Hz, 4-H), 3.30 (1H, dd, $J_{2,3}$=8.4 Hz, 3-H), 3.67 (1H, d, 2-H), 3.69 (1H, dd, $J_{5,8}$=9.2 Hz, J_{gem}=10.6 Hz, 8-H), 3.99 (1H, dd, $J_{5,8'}$=4.0 Hz, 8'-H).

The glycosidase inhibiting activities of **3** and **4** were generally assayed according to the method reported by SAUL *et al.*[11]. While cyclophellitol (**1**) inhibited almond β-glucosidase activity with an IC_{50} of 0.8 μg/ml[1], the α-manno analog **3** expectedly showed inhibitory activity against Jack bean α-mannosidase of IC_{50} 19 μg/ml, indicating that a carba analog of monosaccharides having the proper epoxide ring inhibit the corresponding glycosidase activities as antagonists of the corresponding glycosides. Remarkably, the aziridine analog **4** of cyclophellitol (**1**) showed very high inhibitory activity against almond β-glucosidase of IC_{50} 0.22 μg/ml. This dramatic result suggested that the aziridine-containing polyoxygenated cyclohexanes such as **4** would be stronger glucosidase inhibitors than the epoxide analogs such as **1**. Although further studies are now in progress, the β-galacto analog of **1** was found to inhibit *Escherichia coli* β-galactosidase[12].

† See p. 912.

Acknowledgments

We are grateful to the Institute of Microbial Chemistry for the generous support of our program, and also thank Pharmaceutical Research Laboratories, Meiji Seika Kaisha, Ltd. for an enzyme assay. Financial support by Ministry of Education, Science and Culture (Grant-in-Aid Scientific Research) is gratefully acknowledged.

KUNIAKI TATSUTA
YOSHIHISA NIWATA
KAZUO UMEZAWA
KAZUNOBU TOSHIMA
MASAYA NAKATA

Department of Applied Chemistry,
Keio University,
3-14-1 Hiyoshi, Kohoku-ku,
Yokohama 223, Japan

(Received April 22, 1991)

References

1) ATSUMI, S.; K. UMEZAWA, H. IINUMA, H. NAGANAWA, H. NAKAMURA, Y. IITAKA & T. TAKEUCHI: Production, isolation and structure determination of a novel β-glucosidase inhibitor, cyclophellitol, from *Phellinus* sp. J. Antibiotics 43: 49～53, 1990
2) TATSUTA, K.; Y. NIWATA, K. UMEZAWA, K. TOSHIMA & M. NAKATA: Enantiospecific total synthesis of a β-glucosidase inhibitor, cyclophellitol. Tetrahedron Lett. 31: 1171～1172, 1990
3) TATSUTA, K.; Y. NIWATA, K. UMEZAWA, K. TOSHIMA & M. NAKATA: Total syntheses of glucosidase inhibitors, cyclophellitols. Carbohydr. Res., in press
4) TATSUTA, K.; Y. NIWATA, K. UMEZAWA, K. TOSHIMA & M. NAKATA: Enantiospecific synthesis and biological evaluation of 1,6-*epi*-cyclophellitol. J. Antibiotics 44: 456～458, 1991
5) TONG, M. K. & B. GANEM: A potent new class of active-site-directed glycosidase inactivators. J. Am. Chem. Soc. 110: 312～313, 1988
6) NIWA, T.; S. INOUYE, T. TSURUOKA, Y. KOAZE & T. NIIDA: "Nojirimycin" as a potent inhibitor of glucosidase. Agric. Biol. Chem. 34: 966～968, 1970
7) SCHNEIDER, M. J.; F. S. UNGEMACH, H. P. BROQUIST & T. M. HARRIS: (1S,2R,8R,8aR)-1,2,8-Trihydroxyoctahydroindolizine (swainsonine), an α-mannosidase inhibitor from *Rhizoctonia Leguminicola*. Tetrahedron 39: 29～32, 1983
8) CURRAN, D. P.: Reduction of Δ^2-isoxazolines. 3. Raney-nickel catalyzed formation of β-hydroxy ketones. J. Am. Chem. Soc. 105: 5826～5833, 1983
9) ITTAH, Y.; Y. SASSON, I. SHAHAK, S. TSAROOM & J. BLUM: A new aziridine synthesis from 2-azido alcohols and tertiary phosphines. Preparation of phenanthrene 9,10-imine. J. Org. Chem. 43: 4271～4273, 1978
10) TANNER, D. & P. SOMFAI: From aziridines to carbapenems via a novel β-lactam ring closure. An enantioselective synthesis of (+)-PS-5. Tetrahedron 44: 619～624, 1988
11) SAUL, R.; J. P. CHAMBERS, R. J. MOLYNEUX & A. D. ELBEIN: Castanospermine, a tetrahydroxylated alkaloid that inhibits β-glucosidase and β-glucocerebrosidase. Arch. Biochem. Biophys. 221: 593～597, 1983
12) NIWATA, Y.: Total syntheses of glucosidase inhibitors, cyclophellitols. Ms. Thesis, Keio Univ., 1990