COMMUNICATION TO THE EDITOR

Synthesis and Biological Evaluation of Caloporoside Analogs

Sir:

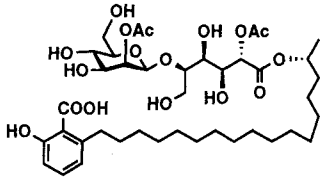
Caloporoside (**1**) is a novel phospholipase C inhibitor isolated from culture filtrates of *Caloporus dichrous*[1], and structurally a salicylic acid derivative containing a β-mannopyranoside. Deacetyl-caloporoside (**2b**) and its analog **3b** have been independently reported to inhibit the binding of ^{35}S-labeled *t*-butylbicyclophosphorothionate (^{35}S-TBPS) to the $GABA_A$/benzodiazepine chloride channel receptor complex *in vitro*[2].

Very recently, we have synthesized deacetyl-caloporoside (**2b**) and its α-mannoside analog (**2a**), and then confirmed the structures of natural products **1** and **2b**[3].

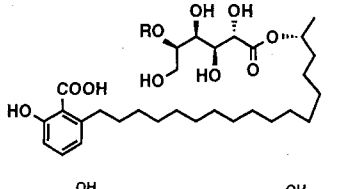
Herein, we describe the synthesis and preliminary biological evaluation of compounds **2a**, **2b**, **3a**, **3b** and **13** to understand the structure-activity relationships.

The syntheses of these compounds originated from the derivatives of D-mannose (**4**), (*R*)-1,3-butanediol (**8**) and salicylic acid (**11**) through glycosylations and Wittig reactions[3].

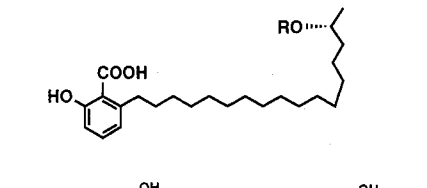
Reaction of the protected mannopyranose **4** with 2-naphthalenethiol gave the 1-thio-mannoside **5** (85%, syrup) corresponding to a glycosyl donor. On the other hand, $NaBH_4$ reduction of **4** followed by selective silylation gave the alcohol **6** corresponding to a glycosyl acceptor. The glycosylation of **5** with **6** was carried out, according to our procedure[3] developed for the synthesis of β-mannopyranosides, in EtOAc with NIS and 0.15 M TfOH in CH_2Cl_2 at −40°C for 1.5 hours to give the corresponding α- and β-mannopyranosides, which were de-*O*-silylated with TBAF and oxidized successively with oxalyl chloride-DMSO and then with sodium chlorite to afford the carboxylic acids **7a** [19% total yield, syrup, $[\alpha]_D$ +8.7° (*c* 1.0, $CHCl_3$)] and **7b** [61% total yield,



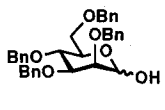
1: Caloporoside



2a: R= α **2b:** R= β (Deacetyl-caloporoside)



3a: R= α **3b:** R= β



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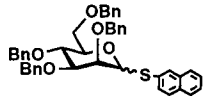

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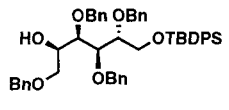

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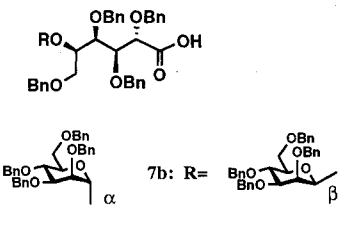
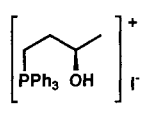


7a: R= α **7b:** R= β



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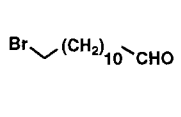

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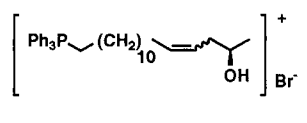

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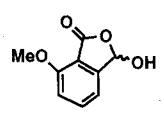

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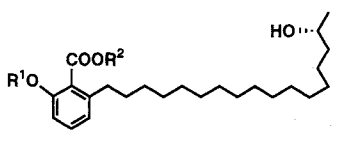
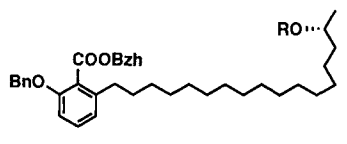


12: R^1= Me, R^2= H
13: R^1= R^2= H
14: R^1= Bn, R^2= Bzh



15a: R= α **15b:** R= β

Table 1. Physico-chemical properties of caloporoside analogs (**2a**, **2b**, **3a**, **3b** and **13**).

No.	$[\alpha]_D$	^1H NMR (ppm)
2a	+18° (*c* 1.0, CH_3OH)	500 MHz (CD_3OD): δ 1.26 (3H, d, J=6 Hz), 2.92 (2H, br t, J=7 Hz), 3.60 (1H, t, J=10 Hz), 3.70 (1H, m), 3.74 (1H, dd, J=10 and 3 Hz), 3.80 (1H, m), 3.87 (1H, dd, J=8 and 1 Hz), 3.88 (1H, dd, J=3 and 2 Hz), 4.21 (1H, d, J=8 Hz), 4.95 (1H, d, J=2 Hz), 4.98 (1H, tq, J=6 and 6 Hz)
2b	−19° (*c* 1.0, CH_3OH)	500 MHz (CD_3OD): δ 1.24 (3H, d, J=6 Hz), 2.95 (2H, br t, J=7 Hz), 3.23 (1H, ddd, J=10, 8 and 2 Hz), 3.44 (1H, dd, J=10 and 3 Hz), 3.49 (1H, t, J=10 Hz), 3.82 (1H, br d, J=9 Hz), 3.87 (1H, ddd, J=9, 6 and 3 Hz), 3.97 (1H, d, J=3 Hz), 4.14 (1H, d, J=9 Hz), 4.72 (1H, s), 4.96 (1H, tq, J=6 and 6 Hz)
3a	+22° (*c* 0.9, CH_3OH)	400 MHz (CD_3OD + acetone *d*-6): δ 1.22 (3H, d, J=6 Hz), 3.11 (2H, br t, J=8 Hz), 3.65 (1H, m), 3.68 (1H, t, J=10 Hz), 3.72 (1H, dd, J=10 and 2 Hz), 3.74 (1H, dd, J=12 and 4 Hz), 3.78 (1H, m), 3.80 (1H, br d, J=2 Hz), 3.81 (1H, dd, J=12 and 2 Hz), 4.88 (1H, br s)
3b	−26° (*c* 1.0, CH_3OH)	270 MHz (CD_3OD): δ 1.14 (3H, d, J=6 Hz), 3.06 (2H, br t, J=8 Hz), 3.20 (1H, ddd, J=10, 5 and 2 Hz), 3.47 (1H, dd, J=10 and 4 Hz), 3.60 (1H, t, J=10 Hz), 3.74 (1H, dd, J=12 and 5 Hz), 3.78 (1H, d, J=4 Hz), 3.86 (1H, dd, J=12 and 2 Hz), 3.91 (1H, m), 4.61 (1H, s)
13	−3.8° (*c* 1.1, CH_3OH)	270 MHz (CD_3OD): δ 1.14 (3H, d, J=6 Hz), 2.88 (2H, m), 3.70 (1H, m)

syrup, $[\alpha]_D$ −29° (*c* 1.0, $CHCl_3$)][3].

The synthesis of the chain portion **14** began with the Wittig reactin of the phosphonium salt **8** and the aldehyde **9** using DMSO-NaH and *n*-BuLi in THF to give the bromo-olefin, which was treated with PPh_3 in MeCN at 80°C for 25 hours to give the other phosphonium salt **10** (90%, syrup)[3]. The second Wittig reaction of **10** with the salicylic acid derivative **11** followed by catalytic reduction afforded the saturated alcohol **12** [64%, mp 80~81°C (toluene), $[\alpha]_D$ −4.2° (*c* 1.1, $CHCl_3$)][3]. This was de-*O*-methylated with LiCl in DMF at 150°C for 3 hours to give **13** [87%, mp 77~79°C (toluene), $[\alpha]_D$ −3.8° (*c* 1.1, MeOH)], which was identical with the naturally derived product in all respects[1,2].

Esterification of **13** with benzophenone hydrazone and HgO to give the benzhydryl ester, followed by benzylation with benzyl bromide and K_2CO_3 in Me_2CO, gave the alcohol **14** [85%, syrup, $[\alpha]_D$ −2.7° (*c* 1.1, $CHCl_3$)].

Coupling of the carboxylic acids **7a** and **7b** with the alcohol **14** was accomplished by the modified Yamaguchi's conditions[3] using 1-naphthoyl chloride to give the corresponding esters, which were submitted to hydrogenolysis in dioxane-aqueous AcOH to give the α-mannoside **2a** and β-mannoside **2b**, respectively (Table 1). **2a**: 58% total yield, syrup, $[\alpha]_D$ +18° (*c* 1.0, MeOH), FAB-MS (*m/z*) 731 $(M-H)^-$. **2b**: 66% total yield, syrup, $[\alpha]_D$ −19° (*c* 1.0, MeOH), FAB-MS (*m/z*) 731 $(M-H)^-$. The physico-chemical properties **2b** were identical with those for natural deacetyl-caloporoside[2].

The direct glycosylation of **5** with the chain portion **14** was also carried out by the aforementioned conditions (NIS, 0.15 M TfOH-CH_2Cl_2 in EtOAc, −40°C, 1 hour) to give the α-mannopyranoside **15a** [18%, syrup, $[\alpha]_D$ +18° (*c* 0.9, $CHCl_3$)] and β-mannopyranoside **15b** [69%, syrup, $[\alpha]_D$ −27° (*c* 0.9, $CHCl_3$)]. The anomeric configurations of **15a** and **15b** were determined by NMR studies, especially based on the direct coupling constants between their anomeric carbons and protons [^{13}C NMR (125 MHz) in $CDCl_3$][4]: $^1J(^{13}CH)$ 168 Hz and 152 Hz, respectively. Hydrogenolysis of **15a** and **15b** with 3.5 atm H_2 and 10% Pd-C in MeOH-$CHCl_3$-AcOH (15:5:1) gave the corresponding acids **3a** and **3b** (Table 1). **3a**: 63%, $[\alpha]_D$ +22° (*c* 0.9, MeOH), FAB-MS (*m/z*) 553 $(M-H)^-$. **3b**: 64%, $[\alpha]_D$ −26° (*c* 1.0, MeOH), FAB-MS (*m/z*) 553 $(M-H)^-$, identical with natural product[2].

Table 2. Inhibitory activities of caloporoside analogs (**2a**, **2b**, **3a**, **3b** and **13**) and the reference compounds in phospholipase C and $GABA_A$ receptor assays.

Assays	IC_{50} (μM/ml)						
	2a	**2b**	**3a**	**3b**	**13**	Neomycin	Muscimol
Phospholipase C	12	12	18	22	16	35	
$GABA_A$	57	39	40	10	ND		2.9×10^{-3}

The inhibitory activities for phospholipase C (rat brain) and the binding of the ligand to the $GABA_A$/benzodiazepine chloride channel receptor complex (rat brain) *in vitro* were generally assayed by Cerep's system according to the methods reported by NAKANISHI[5] and SNODGRASS[6] groups, respectively, as summarized in Table 2. For phospholipase C, [^3H]-phosphatidylinositol-4,5-biphosphate and neomycin were used as the substrate and reference compound[5]. [^3H]-Labeled and unlabeled muscimol were used as the ligand and reference for $GABA_A$ receptor[6].

All caloporoside analogs **2a**, **2b**, **3a** and **3b** were found to show significant biological activities and inhibit

strongly phospholipase C activities in almost same values. In $GABA_A$ receptor ion channel, however, the β-mannoside analogs **2b** and **3b** showed stronger inhibitory activities of the binding of the ligand than their α-analogs **2a** and **3a**. Remarkably, the intact salicylic chain **13** exhibited strong inhibitory activity against phospholipase C, but no activity against the binding of the ligand in $GABA_A$ receptor, suggesting that the chain portion **13** is essential for the appearance of the phospholipase C inhibitory activities at least.

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KUNIAKI TATSUTA*
SHOHEI YASUDA

Graduate School of Science and Engineering,
Advanced Research Center for Science and Engineering,
Waseda University,
3-4-1 Ohkubo, Shinjuku, Tokyo 169, Japan

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