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Novel Calcium Ionophores: Supramolecular Complexation by The Hydroxylated-Bistetrahydrofuran Skeleton of Potent Antitumor *Annonaceous* Acetogenins.

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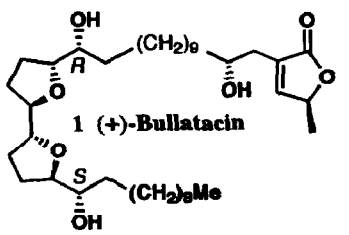
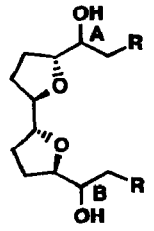
Abstract: Hydroxylated bistetrahydrofuran derivatives which are structural components of potent antitumor *Annonaceous* acetogenins have been revealed to form supramolecular complexes with metal cations. In particular, **2b** formed 2:1 ligand/metal complex with calcium cation with high selectivity.

Annonaceous acetogenins are a new class of natural products with a representative structure as shown in **1**, and have attracted much attention because of a wide variety of biological effects including quite potent and selective antitumor activities.[1] Despite a number of studies on biological effects,[2] structural elucidation,[3] and synthesis,[4] the mode of antitumor activities still remains unclear, and chemical functions of the compounds of this group have not been revealed. Neighboring bistetrahydrofuran skeleton which seems to be an essential part for higher cell growth inhibitory effects might be assumed to be a metal cation binding part from the structural analogy to podands such as oligo-tetrahydropyrans[5] or tetrahydrofurans,[6] however, none of such properties have been reported. We wish to report here the first finding that hydroxylated-bistetrahydrofuran derivatives formed supramolecular complexes with metal cations of biological interest, in particular, **2b** exhibited quite selective binding ability toward calcium cation.





	R=	(A, B)
2a:	$(CH_2)_8CH_3$	(*R*, *R*)
2b:	CH_3	(*R*, *R*)
3a:	$(CH_2)_8CH_3$	(*S*, *R*)
3b:	CH_3	(*S*, *R*)
4a:	$(CH_2)_8CH_3$	(*S*, *S*)
4b:	CH_3	(*S*, *S*)

Hydroxylated-bistetrahydrofuran derivatives with different alkyl chains (R=$(CH_2)_8$Me and R=Me) and relative stereochemistries of *threo-trans-threo-trans-threo* (**2a, b**), *erythro-trans-threo-trans-threo* (**3a, b**) and *erythro-trans-threo-trans-erythro* (**4a, b**) were synthesized starting from diethyl D-tartrate,[7] and were used for metal cation binding experiments. Complexation properties of these model compounds were investigated by ^{1}H-NMR measurements. Most ^{1}H-NMR signals of the compounds were shifted to down fields by the addition of metal salts, among them alcoholic protons exhibited the remarkable changes. Examples of ^{1}H-NMR spectral changes on complexation of **2a**, **2b** and **3a** with $Ca(SCN)_2$ were shown in **Fig. 1**.[8] On the other hand, none of the proton signals of diacetate of **2a** were changed by the addition of metal salts ($Ca(SCN)_2$, NaSCN, or KSCN), indicating that the hydroxy groups play a central role in the binding. Thus, the chemical shift changes of hydroxy protons were subject to the curve fitting method to calculate the complex stability constants (*K*s) and maximum changes of chemical shifts ($\Delta\delta_{max}$).[9] Interestingly, the experimental data were well fitted with the theoretical curves for the ligand-to-cation ratio of 4:1 or 2:1 depending on the ligands and cations. **Fig. 2** showed the comparison of experimental $\Delta\delta$ and theoretical curves

for the complexes of **2a** and **2b** with $Ca(SCN)_2$, and indicated that complexes of 4:1 and 2:1 ligand-to-cation ratio were formed, respectively. Although most of these complex structures have not yet been confirmed, the crystalline complex of **2b** with $Ca(SCN)_2$ was shown to have 2:1 ligand-to-cation ratio by FAB-Mass spectrum.[10] A plausible structure for this complex was generated using the most stable ligand's conformation (**Fig. 3**).[11] It should be noted at this point that integration ratios of shifted hydroxy groups sometimes showed less than unity, as illustrated in **Fig. 1**. As hydroxy groups are attributable to the binding of metal cations, their integration ratios may be regarded as the number of hydroxy groups tightly binding to metal cations. Accordingly, two, four, and four hydroxy groups were shown to be involved in supramolecular complexation of **2a**•Ca^{2+} (4:1), **2b**•Ca^{2+} (2:1) and **3a**•Ca^{2+} (4:1), respectively. The rest hydroxy groups which did not appear in ^{1}H-NMR spectrum were supposed to form hydrogen bonding with each other in the complex or to exchange rapidly with water contaminated in the solvent. Thus, both of the analysis by the curve-fitting method and ^{1}H-integration ratios indicated the formation of supramolecular complexes. **Table 1** summarized binding properties obtained as above.

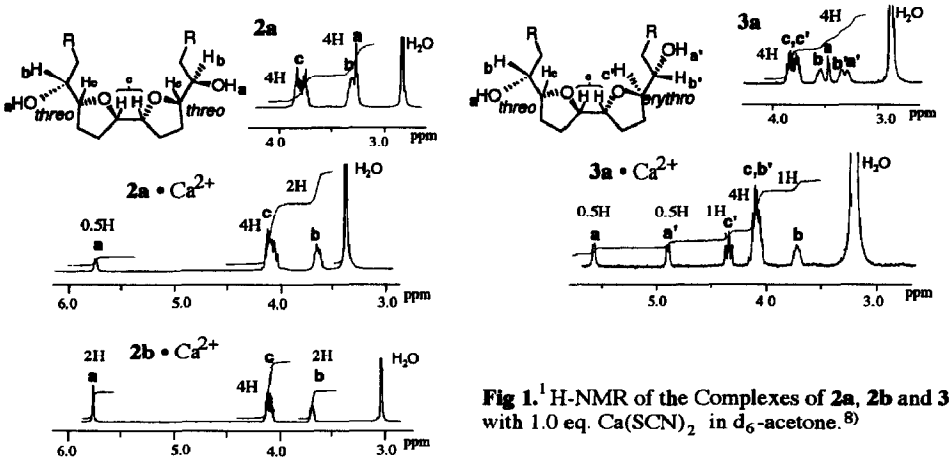


Fig 1. ^{1}H-NMR of the Complexes of **2a**, **2b** and **3a** with 1.0 eq. $Ca(SCN)_2$ in d_6-acetone.[8]

Comparison of **2a** and **2b** showed an interesting contrast in binding potency, Ca^{2+} selectivity, as well as ligand's aggregation states. High selectivity toward Ca^{2+} exhibited by **2b** may be superior to the known ligands, since Ca^{2+} and Mg^{2+} can not be distinguished by the carboxylic antibiotic A23187 (Calcimycin) which is the famous ionophore for divalent cations,[12] and macrocyclic criptands do not discriminate well between Ca^{2+} and Na^+ or K^+.[13] The binding ability of **2b** with Ca^{2+} was shown to be comparable to that of 18-crown-6 from the competition experiment. Ligands with long alkyl chains (**2a**, **3a**, **4a**) formed 4:1 complexes, whereas those with short alkyl chains (**2b**, **3b**, **4b**) formed 2:1 complexes.[14] These results clearly indicates that long alkyl chains assemble ligands into the complex probably due to the van der Waals interactions. It is also noteworthy that effects of the stereochemistry of the hydroxy groups on the binding properties were highly dependent on the ligand's aggregation states. Alteration of metal cation selectivity was also observed in the experiments with ion-selective electrodes containing the ligand in a polymeric membrane, in which the electrode with **2a** or **3a** produced selective response to Ca^{2+} at more than 10^{-5}M, but that with **2b** did not give any responses.[15] Aggregation states of the ligands might be changed in the polymeric membrane. Interestingly, **2a** and **3a** exhibited *in vitro* antitumor activities, but **2b** and **3b** were much less potent.[16]

In conclusion, we revealed that hydroxylated-bistetrahydorfuran derivatives as the model compounds for potent antitumor acetogenins formed supramolecular complexes selectively with calcium cation. The ligands **2b** and **3a** may be useful as neutral calcium ionophores. We speculate,

as Ca^{2+} plays important role in cellular function, that biological activities of acetogenins might be related to Ca^{2+}-binding in the membrane. Theoretical analysis of the origin of calcium selectivity by supramolecular complexes is now in progress.

Table 1. Binding Properties of the Ligands in Solution[a]

Ligand	Metal[b]	Ligand:Metal[c]	*K*s(M^{-1})[d]	Δδmax[d]	No of OH[e]
2a	Ca^{2+}	4:1	149	2.70	2
2a	Mg^{2+}	2:1	85	1.84	4
2a	K^+	4:1	126	1.13	2
2a	Na^+	4:1	44	1.10	2
Ac2-2a	Ca^{2+}	-	0	0	0
3a	Ca^{2+}	4:1	1457	2.59[f]	4[f]
3a	Mg^{2+}	2:1	208	1.44[f]	4[f]
3a	K^+	-	-	g)	-
3a	Na^+	-	-	g)	-
4a	Ca^{2+}	4:1[h]	104	2.16	4
4a	Mg^{2+}	-	-	g)	-
4a	K^+	-	-	g)	-
4a	Na^+	-	-	g)	-
2b	Ca^{2+}	2:1	9020	2.58	4
2b	Mg^{2+}	2:1	40	2.66	4
2b	K^+	-	-	g)	-
2b	Na^+	-	-	g)	-
3b	Ca^{2+}	2:1	830	2.53	2
3b	Mg^{2+}	2:1	62	2.65	2
3b	K^+	-	-	g)	-
3b	Na^+	-	-	g)	-
4b	Ca^{2+}	2:1[h]	47	2.06	2
4b	Mg^{2+}	2:1[h]	68	0.72	2
4b	K^+	-	-	g)	-
4b	Na^+	-	-	g)	-

a) ^{1}H NMR spectra in d_6-acetone were taken at 270 MHz or 500 MHz at different metal salt concentrations. b) Thiocyanates were used. c) The ligand-to-metal ratios which gave the best fit with the experimental data with r^2 values over than 0.999 in the curve-fitting calculation were shown. d) Values were obtained for the complex with the respective ligand/metal ratio. e) The number of hydroxy groups binding to the metal cation in the complex were estimated from the integration values of ^{1}H-NMR spectra. f) Changes of the signal "a" in Fig. 1 were subject to the curve-fitting method. g) Only a little and linear changes of chemical shifts were observed, from which complexation properties could not be calculated. h) The ratios were speculated in analogy with **2a**, **3a** or **2b**, **3b**.

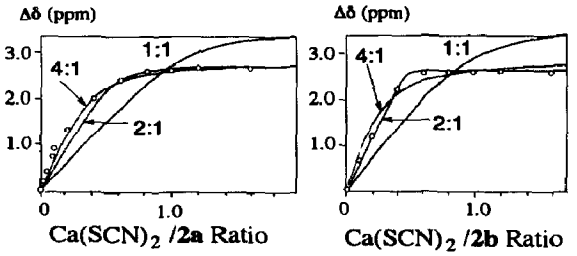


Fig. 2. Experimental Δδ and Theoretical Curves.
Experimental data were shown by circles, and theoretical curves were obtained for supramolecular complexes with the ligand-to-metal ratios indicated.

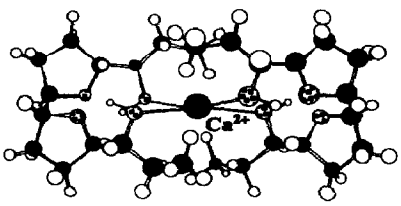


Fig 3. Plausible complex structure of **2b** with Ca^{2+}.
The structure was generated using the most stable lignand's conformation and minimized by ZINDO (CAChe).[11]

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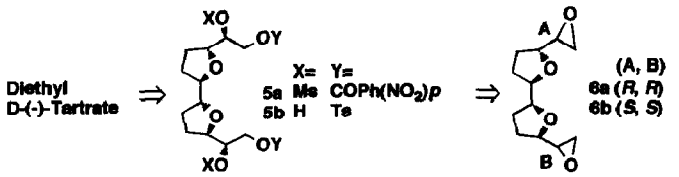
(7) The key intermediates (**5**) were prepared from D-(-)-tartrate. Ligands with C_2 symmetry (**2** and **4**) were obtained from diepoxide (**6a**, **b**), which were derived from dimesylate (**5a**) or ditosylate (**5b**). **3a,b** were synthesized from **2a,b** through stereochemical inversion of one hydroxyl group. All new compounds showed satisfactory IR, ^{1}H-NMR, and high resolution FAB-Mass spectra. Details will be reported elsewhere.



(8) Signals were assigned by H-H COSY spectra.

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(10) Single-crystal X-ray analysis is now in progress. Neutral diamide ligand has been reported to form 1:3 or 1:2 cation/ligand complexes with Ca^{2+} or Mg^{2+}, respectively. see Schefer, U.; Ammann, D.; Pretsch, E.; Oesch, U.; Simon, W. *Anal. Chem.*, **1986**, *58*, 2282.

(11) The most stable conformation of **2b** was searched with MM2 force field, and used to generate the initial complex structure (**2b**:Ca^{2+}= 2:1), which was then minimized by ZINDO. Calculations were performed with CAChe system on Macintosh.

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(14) Mg^{2+} was an exceptional cation in that this cation formed 2:1 complexes with all ligands described here.

(15) The electrodes with **2a**, **2b** or **3a** were prepared with a poly(vinyl chloride)-2-nitrophenyl octyl ether (PVC-NPOE) polymeric membrane, and afforded only poor responses to the cations such as Mg^{2+}, H^+, Li^+, Na^+, or K^+. Details will soon be reported elsewhere.

(16) *In vitro* antitumor activities toward P388 leukemia (IC_{50} [M]): **2a**, 5.6×10^{-7}; **3a**, 2.3×10^{-7}; **2b**, $>1.9\times10^{-4}$; **3b**, $>1.9\times10^{-4}$. These antitumor activities were tested at Exploratory Research Laboratories I, Daiichi Pharmaceutical CO. LTD. Japan, for which we are grateful.

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