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INHIBITORY EFFECTS OF SCOPADULCIC ACID B AND ITS DERIVATIVES ON BONE RESORPTION AND OSTEOCLAST FORMATION *IN VITRO*

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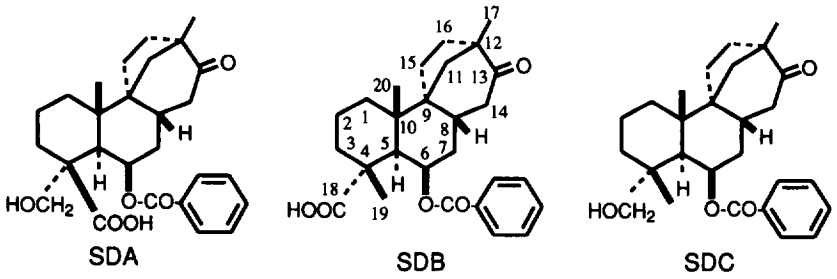
Abstract Scopadulcic acid B and its structurally related compounds showed inhibitory effects on bone resorption and osteoclast formation *in vitro*. Scopadulciol was the most potent inhibitor among 21 compounds tested. Copyright © 1996 Elsevier Science Ltd

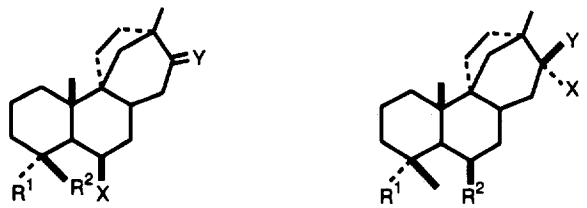
Potentiated bone resorption by osteoclasts is one of the causes of osteoporosis which is characterized by a decrease in bone mass.[1] Osteoclastic bone resorption depends on extracellular acidification of the areas under the ruffled borders. Acid secreted from osteoclasts activates a number of lysosomal enzymes which degrade decalcified bone matrix and cause the solubilization of bone mineral.[2] The major proton transport system of osteoclast is thought to be mediated by a vacuolar-type H^+-ATPase localized at the cell-bone attachment site.[3] The transportation of proton and bone resorption by osteoclasts were inhibited by bafilomicin A1, a specific inhibitor of vacuolar-type H^+-ATPase.[4] In addition, omeprazole and some other inhibitors of gastric H^+,K^+-ATPase, which is different from the vacuolar-type H^+-ATPase, were also found to suppress bone resorption.[5]

In search of biologically active substances from tropical herbal drugs, Hayashi *et al.* isolated unique tetracyclic diterpenoids named scopadulcic acids A (SDA), B (SDB) and scopadulciol (SDC) from *Scoparia dulcis* L. (Scrophulariaceae).[6] The latter two compounds were found to be powerful inhibitors of gastric H^+,K^+-ATPase.[6b,7] From the results of further studies on structure-activity relationships of these ingredients, compounds possessing ether linkages at the position C-6, C-13, and/or C-18 of scopadulan ring system



were revealed to be potent inhibitors of this enzyme.[6b, 8]

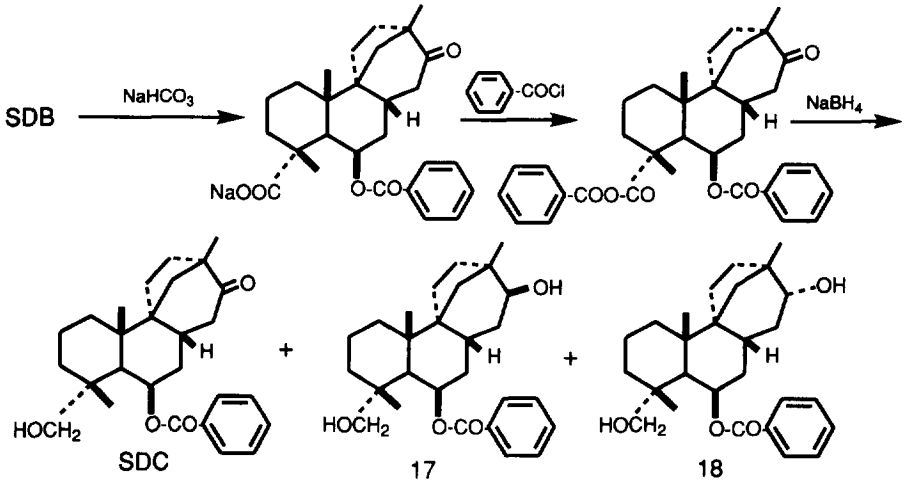
Table 1. List of the compounds tested for ability to inhibit bone resorption



Compound	R^1	R^2	X	Y	Compound	R^1	R^2	X	Y
1	CH_2OH	COOH	OBz	O	**9**	COOH	OBz	H	OH
2	COOH	CH_3	OBz	O	**10**	COOH	OBz	OH	H
3	CH_2OH	CH_3	OBz	O	**11**	COOH	OBz	H	OAc
4	$COOCH_3$	CH_3	OBz	O	**12**	COOH	OBz	OAc	H
5	COOH	CH_3	OH	O	**13**	$COOCH_3$	OBz	H	OH
6	COOH	CH_3	OAc	O	**14**	$COOCH_3$	OBz	OH	H
7	COOH	CH_3	OBz	NOH	**15**	$COOCH_3$	OBz	H	OAc
8	CH_2OAc	CH_3	OBz	O	**16**	$COOCH_3$	OBz	OAc	H
					17	CH_2OH	OBz	H	OH
	OBz = $OCOC_6H_5$				**18**	CH_2OH	OBz	OH	H
	OAc = $OCOCH_3$				**19**	CH_2OH	OH	H	OH
					20	CH_2OH	OH	OH	H
					21	CH_2OAc	OH	H	OAc

In order to evaluate SDB and its derivatives as drug candidates for osteoporosis, a total of twenty one compounds shown in Table 1 were tested for their inhibitory effects on bone resorption in organ culture using neonatal mouse parietal bones. All compounds except for SDA, SDC, and compounds **17** and **18** were prepared by derivatization of SDB or SDC as previously reported by Hayashi *et al.*[6b, 9] SDC was newly obtained

together with compounds **17** and **18** by chemical transformation of SDB according to Scheme 1.[10]



Scheme 1. Chemical transformation of SDB to SDC

Bone-resorbing activity was assesed using the method of Shigeno *et al.*[11] Briefly, ^{45}Ca-prelabeled parietal bones from 4-day-old mice were precultured for 1 day with 1 ml of Eagle minimum essential medium

Table 2. Inhibitory effects of SDB, SDC and their derivatives on PTH-stimulated bone resorption

Compound	Inhibition (%)[a]			Compound	Inhibition (%)[a]		
	2 μM	5 μM	10 μM		2 μM	5 μM	10 μM
1	46	24	-14	**12**	27	26	42
2	28	76	94	**13**	-23	-17	-10
3	106	99	99	**14**	22	29	49
4	78	74	110	**15**	38	51	55
5	43	92	94	**16**	55	40	37
6	33	40	47	**17**	-51	99	-
7	28	66	90	**18**	-60	95	-
8	12	107	125	**19**	-5	-25	-4
9	35	39	70	**20**	-8	6	-17
10	45	40	37	**21**	1	23	11
11	21	18	8				

[a] Inhibition (%) = 100 % - Bone resorption (%) of each compound. Bone resorption (%) = [^{45}Ca release (%) in the presence of PTH and compound - ^{45}Ca release (%) in the absence of PTH]; ^{45}Ca release (%) = (dpm in medium)/(dpm in medium + dpm in bone). - : not tested.

(MEM) containing 5% horse serum and then cultured for 6 days with the medium in the presence of parathyroid

hormone (PTH) and a test compound. An aliquot of the medium and the bone extract was used to count the radioactivity. Table 2 summarizes the effects of the test compounds on PTH-stimulated bone resorption. Naturally occurring SDB (**2**) and SDC (**3**) significantly inhibited the bone resorption at concentrations over 5 μM. In addition, the compounds **4**, **5**, **8**, **17** and **18**, derived from SDB or SDC, also showed potent inhibitory activity. SDC and methyl ester of SDB (**4**) kept their potency at lower concentration (2 μM). On the other hand, other active compounds lost their activity at this concentration. SDC was the most potent inhibitor of bone resorption among the 21 compounds tested. Figure 1 illustrates the dose-responce curves of SDB and SDC. SDB inhibited the bone resorption dose-dependently at the concentration range of 2 to 10 μM, while SDC showed a complete inhibition at higher than 0.5 μM. These experimental results indicate that inhibitory activities of the test compounds on bone resorption do not always agree with those on gastric H^+,K^+-ATPase.[6b]

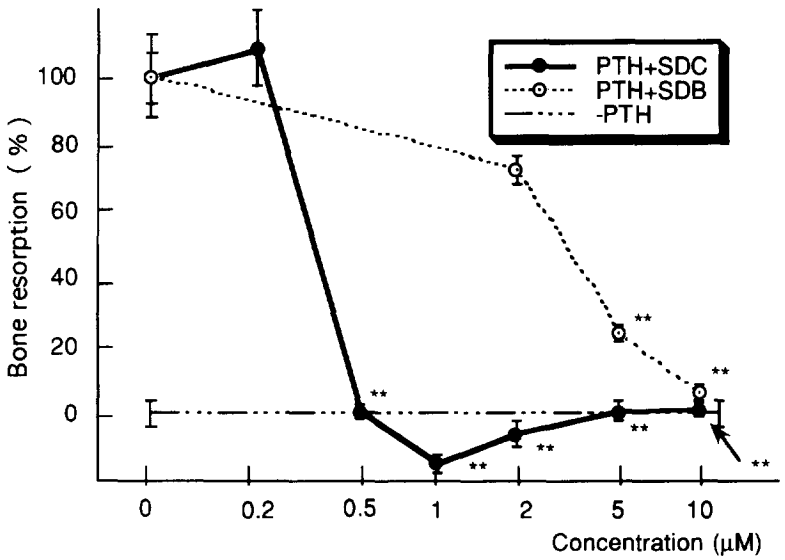


Figure 1. Effects of SDB and SDC on ^{45}Ca release stimulated by PTH.
$^{**}p < 0.01$ vs PTH alone.

To investigate the mechanism of action of SDC and some of its derivatives on bone resorption, we tested the effect of the drugs on osteoclast-like cell formation according to the method reported by Takahashi *et al.*[13] Briefly, mouse osteoblastic cells (1.8 x 10^4 cells/well) and mouse bone marrow cells (2 x 10^5 cells/well) were co-cultured for 6 days in α-MEM containing 10% fetal calf serum and PTH in 24-well culture plates.

Table 3. Inhibitory effects of SDB (**2**), SDC (**3**) and their derivatives on PTH-stimulated osteoclast-like cell formation

Compound	Inhibition (%)[a] 2 μM	5 μM	10 μM	Compound	Inhibition (%)[a] 2 μM	5 μM	10 μM
2	59	74	87	**10**	17	87	104
3[b]	137	137	137	**12**	55	72	79
4	70	91	118	**15**	81	109	134
5	0	10	20	**16**	60	70	112
6	-8	27	37	**17**	24	75	94
8	45	90	105	**18**	17	87	105
9	24	75	93				

[a] Inhibition (%) = 100 % - Osteoclast formation (%) of each compound.

[b] Inhibition (%) of **3** at 0.5, 0.1, and 0.05 μM are 137, 115, and 44 %, respectively.

Tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (MNC) containing three or more nuclei were counted as osteoclast-like cells.[13] As indicated in Table 3, all compounds except for **5** and **6** dose-dependently inhibited PTH-induced TRAP-positive MNC formation. Especially, SDC showed complete inhibition at concentration over 0.1 μM.

It is known that the multinucleated osteoclasts are primary cells responsible for bone resorption and bone resorption is enhanced by two different mechanisms: one is the recruitment of osteoclasts and the other is the activation of resting osteoclasts.[14] As SDC and its derivatives inhibited the osteoclast-like cell formation, inhibitory effects of these diterpenoids on bone resorption may be at least partly due to the inhibition of osteoclast formation.

The results of this study suggest that SDB and its derivatives, especially, SDC, could be promising candidates of therapeutic drugs for osteoporosis. *In vivo* study for further pharmacological evaluation of these compounds is currently underway.

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