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SILYL ETHERS OF CYCLOHEPTENE, NOVEL PROTON PUMP INHIBITORS OBTAINED DURING THE TOTAL SYNTHESIS OF THE SCOPADULCIC ACIDS

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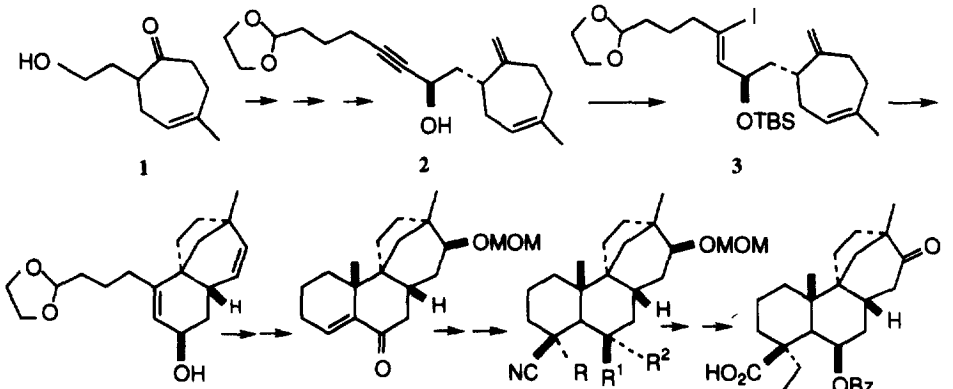
Abstract Synthetic compounds with the scopadulan ring system showed inhibitory effects on the gastric proton pump when they have ether linkages at positions C-6, C-13 and/or C-18. *Tert*-butyldimethylsilyl ether of 5-methylenecycloheptene and related compounds revealed to be novel proton pump inhibitors.

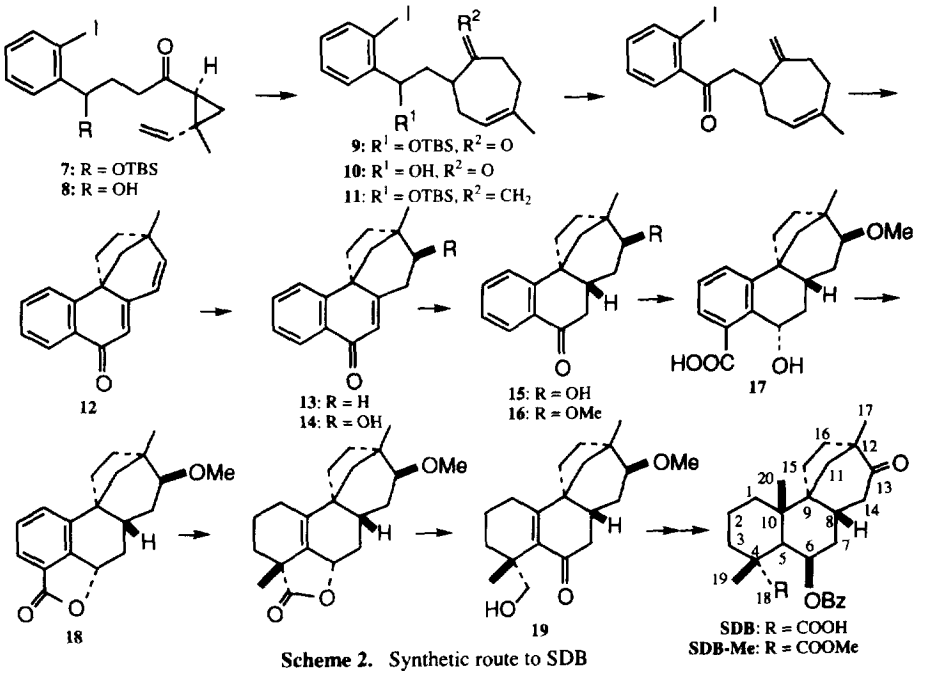
Gastric H^+,K^+-ATPase, the proton pump of the parietal cell, engages in the terminal step of acid secretion in the stomach.[1] Inhibition of this enzyme results in the reduction of gastric acid secretion induced by any of three key messengers: histamine, acetylcholine and gastrin.[2] Omeprazole, one of the synthetic proton pump inhibitors, has been clinically used with splendid results for the treatment of gastric and duodenal ulcers.[3]

In our search for biologically active substances from the Paraguayan crude drug "Typycha kuratu" (*Scoparia dulcis* L., Scrophulariaceae), we isolated a powerful proton pump inhibitor, scopadulcic acid B (SDB) which is a structurally unique tetracyclic diterpenoid.[4] Recently, Overman *et al.* accomplished the total



Scheme 1. Synthetic route to SDA

4: R = H, R^1, R^2 = O
5: R = H, R^1 = OTMS, R^2 = H
6: R = CH_2OCH_2Ph, R^1 = OTMS, R^2 = H



Scheme 2. Synthetic route to SDB

syntheses of racemic SDB as well as scopadulcic acid A (SDA).[5] During the course of these synthetic studies, various reaction products were obtained (Scheme 1 and 2). The desire to find novel proton pump inhibitors led us to examine the effect of these synthetic intermediates on hog gastric H^+,K^+-ATPase. A total of nineteen compounds were tested and the results are summarized in Table 1. Among eleven compounds with the scopadulan ring system, compounds **5** and **6** were found to be effective inhibitors, comparable to SDB.

Table 1. Inhibitory activity of compounds **1** - **19**, **SDB** and **SDB-Me** against the gastric H^+,K^+-ATPase activity

Compound	Inhibition % 1 μM	10 μM	100 μM	Compound	Inhibition % 1 μM	10 μM	100 μM
1	11	9	34	**12**	16	9	61
2	8	13	20	**13**	-2	9	52
3	14	63	93	**14**	8	17	17
4	5	7	17	**15**	18	19	0
5	4	32	67	**16**	-12	17	42
6	25	44	52	**17**	-6	0	21
7	29	50	80	**18**	1	16	55
8	8	35	63	**19**	9	14	8
9	30	74	95	**SDB**	7	21	62
10	10	19	72	**SDB-Me**	36	74	81
11	15	63	98				

Both active compounds possess ether linkages at C-6, C-13 and/or C-18. These findings agree with our previous results [6] suggesting that relatively longer side chain substituents on the scopadulan ring system enhance inhibition of the proton pump.

Intramolecular Heck bis-cyclizations of 5-methylenecycloheptene were central steps in the construction of the BCD ring system of the scopadulcic acids.[5] In the synthesis of SDB, compound **9**, a Δ^4cycloheptenone containing a 2-aryl-2-(siloxy)ethyl side chain, was prepared prior to the key intramolecular Heck reaction. When this compound was assayed, it strongly inhibited the proton pump. Interestingly, all silyl ethers in this series (**3**, **7**, **9** and **11**) were potent inhibitors, while the parent alchols were less active (Table 1). Compound **9** was the most potent inhibitor among 19 compounds tested. As shown in Figure 1, this compound inhibited gastric H^+,K^+- ATPase activity dose-dependently (IC_{50} = 8.2 μM). The inhibition potency of **9** was almost the same as that of the methyl ester of SDB (SDB-Me), the most potent inhibitor among semisynthetic derivatives of SDB.[6]

A kinetic study was carried out to investigate the mechanism by which **9** inhibits H^+,K^+-ATPase. When the effect of **9** on the H^+,K^+-ATPase activity was measured in the presence of various concentrations of K^+, the double-reciprocal (Lineweaver-Burk) plots illustrated in Figure 2 were obtained. The plots in

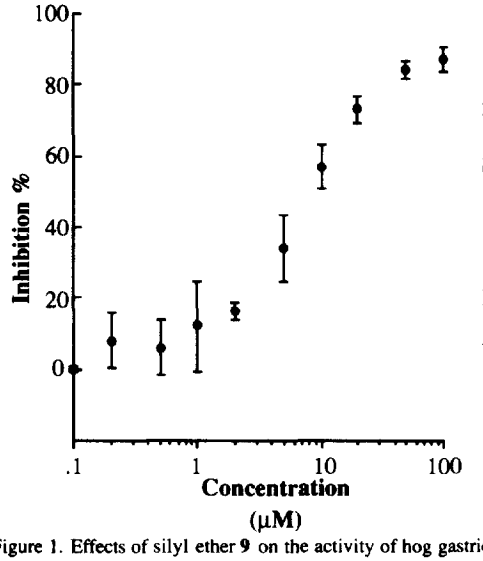


Figure 1. Effects of silyl ether **9** on the activity of hog gastric H^+,K^+-ATPase. Data shown are averages±s.e. for three experiments.

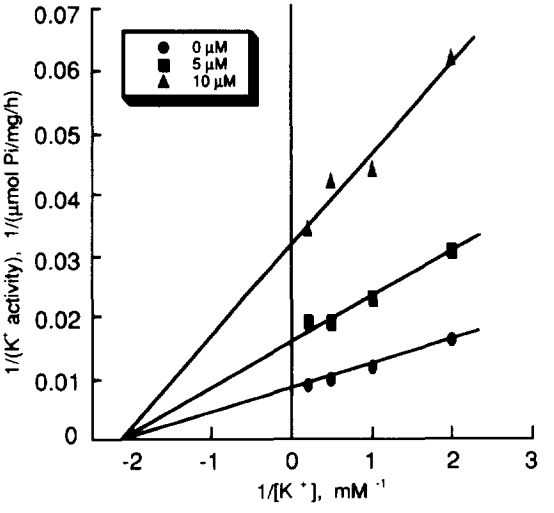


Figure 2. Lineweaver-Burk plots between the K^+-ATPase activity in lyophilized vesicles and the medium concentration of K^+ (0.5-10 mM). Typical results of one experiment are shown.

Figure 2 show that the inhibition of H^+,K^+-ATPase activity by **9** is non-competitive with respect to K^+. The apparent V_{max} value was changed from 125 to 63 and 31 μmole/hr/mg protein in the presence of 0, 5 and 10 μM of silyl ether **9**, respectively, while the apparent Km value remained unchanged (Km = 0.46 mM). This inhibition pattern is different from those of SDB (mixed type), diacetyl scopadol (DAS) (uncompetitive type) and SCH-28080 (competitive type).[4b,7] So far, several naturally occurring phenolic compounds such as salvianolic acid A, cassigarol A and piceatanol have been reported to be non-competitive inhibitors of gastric H^+,K^+-ATPase with respect to K^+.[8] Compound **9** and related silyl ethers might be useful tools for molecular biological studies of the gastric proton pump, since they are structurally quite distinct from all previously described inhibitors.

In recent years, some gastric proton pump inhibitors were reported to inhibit bone resorption due to the presence of a H^+,K^+-ATPase like proton pump in osteoclasts.[9] SDB was also found to inhibit bone resorption by osteoclast cells.[10] Therefore, further experimental studies of these active compounds on gastric acid secretion *in vivo* and also of osteoclast-mediated bone resorption will allow evaluation of these compounds as possible therapeutics for treating ulcer and osteoporosis, respectively.

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