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SCOPADULCIOL, AN INHIBITOR OF GASTRIC H+, K+-ATPASE FROM *SCOPARIA DULCIS*, AND ITS STRUCTURE-ACTIVITY RELATIONSHIPS

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ABSTRACT.—A new tetracyclic diterpenoid, scopadulciol [3], together with 6-methoxybenzoxazolinone, glutinol, and acacetin, was isolated from the 70% EtOH extract of *Scoparia dulcis* collected in Taiwan. Its structure was elucidated to be 6β-benzoyl-12-methyl-13-oxo-9(12)$_a$,9(12)$_b$-dihomo-18-podocarpanol on the basis of spectral data. It mildly inhibited hog gastric H+,K+-ATPase.

Examination of the inhibitory activities of derivatives of scopadulcic acid B [2], including 3, revealed that methylation of the carboxyl group and introduction of an acetyl group or oxime at C-13 or C-18 markedly enhanced the inhibitory activity, while debenzoylation reduced the activity. Among the 30 compounds tested, compound 12, a methyl ester of scopadulcic acid B [2], showed the most potent activity.

The enzyme H+,K+-ATPase is an electroneutral H+/K+ exchange pump responsible for the secretion of acid into the stomach (1). This ATPase was found to be localized in the tubulovesicular and secretory membranes of the acid-producing parietal cell (2,3). Recently, SCH28080 {2-methyl-8-(phenylmethoxy)imidazo[1,2a]pyridine-3-acetonitrile} and some substituted benzimidazoles such as omeprazole have been shown to be novel antiulcer agents with antisecretory activity (4–6). The inhibition of acid formation by these compounds has been proposed to be due to their ability to block H+,K+-ATPase (7–9).

In our studies on biologically active substances from a Paraguayan crude drug, "Typychá kuratū" (*Scoparia dulcis* L., Scrophulariaceae), we have isolated the cytotoxic tetracyclic diterpenoids named scopadulcic acids A (SDA) [1] and B (SDB) [2] (10). SDB was found to inhibit gastric acid secretion (11), as well as replication of herpes simplex virus type 1 (12). The inhibition mechanism of acid secretion was clarified to involve a direct inhibition of gastric pump, the H+,K+-ATPase (13,14). Here, we report the isolation of a new SDB analogue scopadulciol [3] from *S. dulcis* collected in Taiwan, which mildly inhibited gastric H+,K+-ATPase. The present paper describes the structure elucidation of scopadulciol [3] and the inhibitory activities of a series of its derivatives against H+,K+-ATPase.
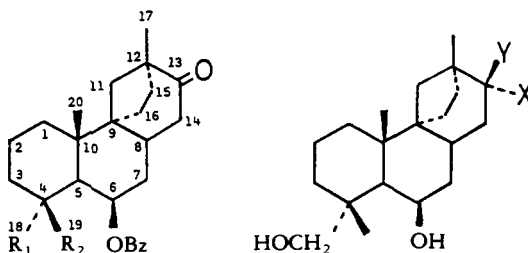
Bz = COC₆H₅

1 R₁=CH₂OH, R₂=COOH   5 X=OH, Y=H
2 R₁=COOH, R₂=Me   6 X=H, Y=OH
3 R₁=CH₂OH, R₂=Me
4 R₂=CH₂OAc, R₂=Me

RESULTS AND DISCUSSION

The dried, powdered leaves and twigs of *S. dulcis* were extracted with hot 70% EtOH. The 70% EtOH extract was partitioned between H_2O and $CHCl_3$. The $CHCl_3$-soluble part was further purified by cc to yield scopadulciol [3] (0.035% yield), together with 6-methoxybenzoxazolinone (15), glutinol (16), and a flavonoid, acacetin (17). This is the first report of the isolation of acacetin from *S. dulcis*.

Compound 3, $C_{27}H_{36}O_4$, $[\alpha]^{23}D - 2.3°$ ($c = 0.5$, $CHCl_3$), was obtained as a colorless amorphous powder. The ir and uv spectra showed absorption maxima at 3570 (OH), 1710 (CO), 1695 (CO), 1600, and 1585 (phenyl) cm^{-1} and 229 (4.08), 267 sh (2.85), 272 (2.92), and 280 (2.85) nm, respectively. The eims spectrum showed fragment ion peaks at m/z 319 $[M - C_6H_5CO]^+$, 302 $[M - C_6H_5COOH]^+$, 122 $[C_6H_5COOH]^+$, 105 $[C_6H_5CO]^+$, and 77 $[C_6H_5]^+$, indicating the presence of a benzoyl group. The ^1H-nmr spectrum of 3 showed the presence of three tertiary methyl groups (δ 0.92, 1.09, 1.54), a hydroxymethylene group (δ 3.11 and 3.58, each 1H, d, $J = 11.0$ Hz), an oxymethine proton (δ 5.68, 1H, brd, $J = 2.2$ Hz), and a monosubstituted benzene ring (δ 7.46, 2H, d, $J = 7.3$ Hz; 7.57, 1H, t, $J = 7.3$ Hz; 8.03, 2H, t, $J = 7.3$ Hz). Acetylation of 3 with Ac_2O in pyridine gave a monoacetate 4 and caused the downfield shift of a pair of doublets at δ 3.11 and 3.58 to δ 3.74 and 3.95, respectively. These spectral data suggest that 3 is an SDB analogue. As shown in Table 1, the ^{13}C-nmr spectral data of 3 are quite similar to those of SDB (10) except for the presence of an oxymethylene carbon signal at δ 71.2 instead of the carboxyl carbon signal at δ

TABLE 1. ^{13}C-nmr Data of Scopadulcic Acid B [2] and Scopadulciol [3] in $CDCl_3$.

Carbon	Compound	
	2	3
C-1	34.0	34.3
C-2	18.0	18.1
C-3	39.7	38.4
C-4	47.2	37.7
C-5	44.6	43.1
C-6	72.9	70.3
C-7	35.1	35.4
C-8	36.0	35.9
C-9	53.1	53.0
C-10	38.8	39.0
C-11	45.1	45.5
C-12	52.3	52.3
C-13	213.6	213.7
C-14	42.5	42.7
C-15	23.7	23.7
C-16	36.6	36.7
C-17	19.7	20.5
C-18	184.2	71.2
C-19	19.3	19.8
C-20	21.6	21.8
C-21	166.1	166.4
C-1'	130.5	130.7
C-2', -6'	129.6	129.7
C-3', -5'	128.5	128.6
C-4'	133.4	133.1

184.2 in SDB. These data are consistent with structure **3**. Chemical correlation was proved by LiAlH$_4$ reduction of **3** to afford triols **5** and **6** which were also obtained from SDB by reduction with LiAlH$_4$.
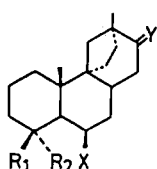
The absolute configuration of **3** was determined on the basis of cd spectral data. It displayed a positive Cotton effect at 297 nm ([θ] +5198) due to the ketone chromophore at the C-13 position, indicating the absolute configuration of scopadulciol to be depicted as in **3**. Thus, scopadulciol [**3**] was characterized as 6β-benzoyl-12-methyl-13-oxo-9(12)$_a$,9(12)$_b$-dihomo-18-podocarpanol.

When the inhibitory activities of compounds **3–6** against H$^+$,K$^+$-ATPase were tested, **3** and **4** were found to be active, while **5** and **6** were inactive. Therefore, a series of SDB derivatives were examined for their inhibitory activities on this enzyme and some structure-activity relationships were studied.

As previously reported (18), the ketone at the C-13 position of SDB was reduced to a hydroxyl group with NaBH$_4$ or LiAlH$_4$, and the carboxyl group at C-4 was esterified with CH$_2$N$_2$ in Et$_2$O, or reduced to a hydroxyl group with LiAlH$_4$. The benzoyl group at the C-6 position was modified to a hydroxyl group by hydrolysis with KOH in DMSO/MeOH and the product then oxidized to a ketone with Jones reagent. The newly formed hydroxyl group at the C-13 or C-18 position was transformed to an acetyl group with Ac$_2$O/pyridine at room temperature. The hydroxyl group at the C-6 position was acetylated by treating with Ac$_2$O and 4-dimethylaminopyridine in pyridine. The sodium salt of SDB was obtained as reported elsewhere (13). In addition to these compounds, oxime **14** and lactone **16** were prepared as described in the Experimental section.

The inhibitory activities of scopadulciol [**3**] and its derivatives against hog gastric H$^+$,K$^+$-ATPase were evaluated, and the results are shown in Tables 2 and 3. Modifica-
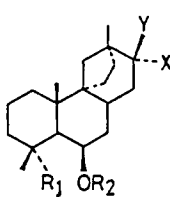
TABLE 2. Inhibitory Activities of Scopadulciol [**3**] and Its Derivatives Against Gastric H$^+$,K$^+$-ATPase.

Compound	R$_1$	R$_2$	X	Y	Inhibition %		
					1 μM	10 μM	100 μM
1	COOH	CH$_2$OH	OBz[a]	O	—	—	24
2	Me	COOH	OBz	O	7	21	62
3	Me	CH$_2$OH	OBz	O	−1	21	45
4	Me	CH$_2$OAc	OBz	O	40	71	72
7	Me	COOH	OH	O	b	b	b
8	Me	COOH	OAc	O	1	7	33
9	Me	COOMe	OH	O	−5	4	52
10	Me	COOH	O	O	−1	1	12
11	Me	COOMe	O	O	b	b	b
12	Me	COOMe	OBz	O	36	74	81
13	Me	COONa	OBz	O	0	14	64
14	Me	COOH	OBz	NOH	11	26	80
15	Me	COOMe	OBz	NOH	24	67	75
16	Me	CO	O	O	2	11	19

[a]Bz = COC$_6$H$_5$.
[b]Insoluble in reaction medium.

TABLE 3.   Inhibitory Activities of Scopadulciol Derivatives Against Gastric H^+,K^+-ATPase.

Compound					Inhibition %		
	R_1	R_2	X	Y	1 μM	10 μM	100 μM
5	CH_2OH	H	OH	H	−11	−3	7
6	CH_2OH	H	H	OH	—	—	24
17	CH_2OAc	H	OAc	H	−2	33	62
18	CH_2OAc	H	H	OAc	5	40	79
19	CH_2OAc	Ac	H	OAc	13	18	57
20	COOH	H	H	OH	—	—	8
21	COOH	H	H	OAc	2	13	21
22	COOMe	H	H	OH	−2	5	22
23	COOH	COC_6H_5	OH	H	−4	3	30
24	COOH	COC_6H_5	H	OH	1	12	72
25	COOH	COC_6H_5	OAc	H	7	38	98
26	COOH	COC_6H_5	H	OAc	3	21	94
27	COOMe	COC_6H_5	OH	H	12	59	64
28	COOMe	COC_6H_5	H	OH	10	64	68
29	COOMe	COC_6H_5	OAc	H	35	58	61
30	COOMe	COC_6H_5	H	OAc	49	61	62

tion of the functional groups at the C-4, C-6, and C-13 positions influenced the activity remarkably. The characteristic tendency was as follows: (1) methylation of the carboxyl group at the C-4 position enhanced the activity (**2**<**12**, **14**<**15**, **20**<**22**, **23**<**27**, **24**<**28**); (2) debenzoylation at the C-6 position reduced the activity (**12**>**9**, **24**>**20**, **26**>**21**, **28**>**22**); (3) introduction of an acetyl group or an oxime at the C-13 or C-18 positions led to an increase in the potency of inhibition (**2**<**14**, **3**<**4**, **5**<**17**, **6**<**18**,
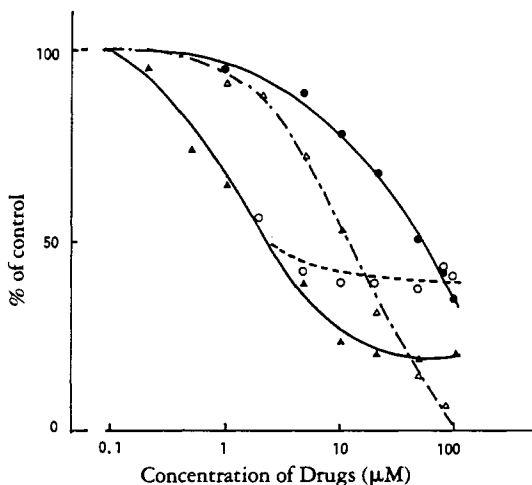

FIGURE 1.   Effect of scopadulcic acid B [2] and its derivatives **12**, **25**, and **29** on H^+,K^+-ATPase: ————●———— **2**, ————▲———— **12**, — ·-△-· — **25**, – –○– – **29**.

$20<21$, $24<26$); (4) compounds with poor H_2O solubility produced a lag in activity (4, 12, 27, 28, 29, 30). They showed relatively more potent activity at lower concentration. As shown in Table 3, the configuration at C-13 had no influence on activity. Figure 1 illustrates the relationship between activity and concentration of 2, 12, 25, and 29. Since compounds 12 and 25 exerted a strong effect on the activity of H^+,K^+-ATPase, the hybrid compound 29 was expected to show more potent activity. The activity of 29 was, however, less potent than that of the parent compounds at higher concentration. This result is explainable by a decrease of solubility in H_2O (Figure 2). The sample solution of this compound became turbid at higher concentrations. The 50% inhibitory concentration (IC_{50}) of 12 and 29 is 3–5 μM. This value is similar to that of omeprazole (19) and SCH28080 (20). In summary, a series of SDB derivatives, especially compounds possessing an ester group at the C-4, C-6, or C-13 positions, were found to be good inhibitors of gastric H^+,K^+-ATPase. Among the 30 compounds tested, 12 was the most potent inhibitor and may be a candidate for a new type of antiulcer agent.
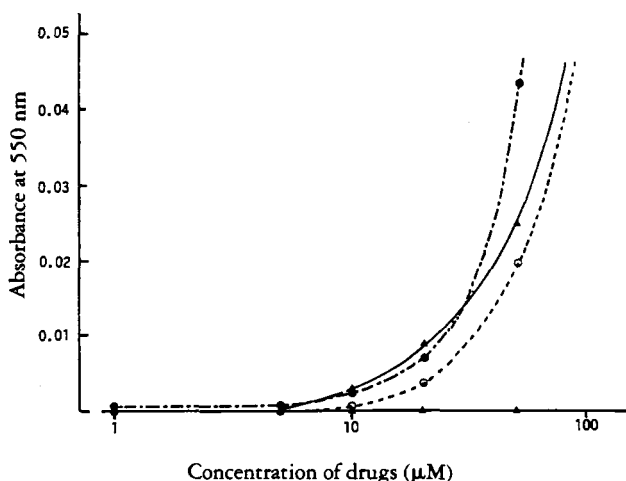

FIGURE 2. Relationship between concentration of drugs and turbidity. — · —●— · — 4, ———▲——— 12, ———△——— 25, – – –○– – 29.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ir spectra were taken on a Hitachi 260-10 infrared spectrophotometer. Uv spectra were recorded on a Hitachi 220S double beam spectrophotometer. ^1H- and ^{13}C-nmr spectra were determined with a JEOL GX-270 spectrometer (270 MHz) and Varian XL-200 spectrometer (50.3 MHz), respectively, and chemical shifts are given in ppm with TMS as an internal standard. Eims were obtained with a JEOL JMS-D200 mass spectrometer. Specific rotations were recorded on a JASCO DIP-140 digital polarimeter. Cd spectrum was recorded on a JASCO J-500 spectropolarimeter. Si gel was used for cc (MERCK Kieselgel 60, 70–200 mesh) and tlc (Wako Si gel 70F$_{254}$ Plate).

PLANT MATERIAL.—*S. dulcis* was collected and identified in August 1988 from Kaohsiung Hsien, Taiwan by Dr. C.N. Lin. A voucher specimen has been deposited in the herbarium of our university.

ISOLATION PROCEDURE.—The ground, air-dried leaves and twigs of *S. dulcis* (585 g) were extracted three times with 70% EtOH at boiling temperature for 1 h. The combined extracts were concentrated, and the aqueous residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble part was evaporated to give a dark brown residue (33.5 g), a portion of which (30.0 g) was subjected to cc on Si gel (600 g, 7 × 35 cm). Elution with $CHCl_3$ afforded a fraction (4.9 g) which contained 3, as well as 6-methoxybenzoxazolinone, glutinol, and acacetin. These compounds were separated and purified by repeated cc on Si gel using *n*-hexane/$CHCl_3$ or $CHCl_3$/MeOH as eluting solvent. Yield: 6-methoxybenzoxazolinone, 13.8 mg

(0.0023%); glutinol, 8.2 mg (0.0014%); acacetin, 6.5 mg (0.0011%); scopadulciol [3], 206.7 mg (0.035%). 6-Methoxybenzoxazolinone, glutinol, and acacetin were identified by direct comparison with authentic samples.

SCOPADULCIOL [3].—Compound 3 was obtained as a colorless amorphous powder: $[\alpha]^{23}$D $-2.3°$ ($c = 0.5$, CHCl$_3$); ir ν max (KBr) 3570, 1710, 1695, 1600, 1585 cm^{-1}; uv λ max (MeOH) (log ϵ) 229 (4.08), 267 sh (2.85), 272 (2.92), 280 (2.85) nm; cd $[\theta]_{297}$ (CHCl$_3$) $+5198$; ^{1}H nmr (CDCl$_3$) δ 0.92 (3H, s, Me-17), 1.09 (3H, s, Me-19), 1.54 (3H, s, Me-20), 2.50 (1H, m, H-8), 3.11 and 3.58 (each 1H, d, $J = 11.0$ Hz, H-18), 5.68 (1H, br d, $J = 2.2$ Hz, H-6), 7.46 (2H, t, $J = 7.3$ Hz, H-3', -5'), 7.57 (1H, t, $J = 7.3$ Hz, H-4'), 8.03 (2H, d, $J = 7.3$ Hz, H-2', -6'); ^{13}C nmr (CDCl$_3$) see Table 1; ms *m/z* [M + 1]$^+$ 425, [M $-$ Me]$^+$ 409, [M $-$ C$_6$H$_5$CO]$^+$ 319, [M $-$ C$_6$H$_5$COOH]$^+$ 302, [C$_6$H$_5$COOH]$^+$ 122, [C$_6$H$_5$CO]$^+$ 105 (base), [C$_6$H$_5$]$^+$ 77; hrms *m/z* [M $-$ 1]$^+$ 423.2460 (calcd for C$_{27}$H$_{35}$O$_4$, 423.2533). *Anal.* calcd for C$_{27}$H$_{36}$O$_4$·H$_2$O, C 73.26, H 8.66; found C 73.49, H 8.42.

ACETYLATION OF 3.—Compound 3 (31 mg) was dissolved in pyridine (0.5 ml), and Ac$_2$O (0.5 ml) was added. After leaving the reaction mixture at room temperature for 15 h, it was poured into ice-H$_2$O and extracted with EtOAc. The EtOAc-soluble portion (31 mg) was subjected to cc on Si gel to give 4 (11 mg) as colorless prisms from CHCl$_3$/MeOH: mp 167–169°; $[\alpha]^{23}$D $-14.9°$ ($c = 0.5$, CHCl$_3$); ir ν max (KBr) 1730, 1710, 1600, 1580 cm^{-1}; uv max (MeOH) (log ϵ) 229 (4.10), 267 sh (2.85), 272 (2.91), 280 (2.82) nm; ^{1}H nmr (pyridine-d_5) δ 1.05 (3H, s, Me-17), 1.09 (3H, s, Me-19), 1.54 (3H, s, Me-20), 2.25 (1H, s, OAc), 2.47 (1H, m, H-8), 3.74 and 3.95 (each 1H, d, $J = 11.0$ Hz, H-18), 5.61 (1H, br d, $J = 2.4$ Hz, H-6), 7.46 (2H, t, $J = 7.4$ Hz, H-3', -5'), 7.58 (1H, t, $J = 7.4$ Hz, H-4'), 8.04 (2H, d, $J = 7.4$ Hz, H-2', -6'); hrms *m/z* [M]$^+$ 466.2736 (calcd for C$_{29}$H$_{38}$O$_5$, 466.2717). *Anal.* calcd for C$_{29}$H$_{38}$O$_5$, C 74.63, H 8.21; found C 74.28, H 8.31.

REDUCTION OF 3 WITH LiAlH$_4$.—LiAlH$_4$ (30 mg) was added to a solution of 3 (26 mg) in dry Et$_2$O (5 ml) under stirring at room temperature. Stirring was continued for 1 h, and the mixture was treated as described previously (11). The reaction products were chromatographed on a Si gel column using CHCl$_3$ as the eluting solvent. The first eluate was evaporated and the residue recrystallized from CHCl$_3$/MeOH to give colorless needles of 5 (5 mg). The second eluate was evaporated to yield a colorless amorphous powder of 6 (5 mg). The products 5 and 6 were identified as 12-methyl-9(12)$_a$,9(12)$_b$-dihomo-6β,13α,18-podocarpanetriol and 12-methyl-9(12)$_a$,9(12)$_b$-dihomo-6β,13β,18-podocarpanetriol, respectively, by comparison of their spectral data with those of authentic samples.

DERIVATIZATION OF SCOPADULCIC ACID B [2].—Compounds 7–13, 17, 18, and 20–28 were obtained by derivatization of 2 as described elsewhere (13, 18).

PREPARATION OF OXIME 14.—KOH (200 mg) in MeOH (11.5 ml) was added dropwise over 45 min to a solution of 2 (100 mg) and NH$_2$OH·HCl (100 mg) in 95% EtOH (12 ml) at 50°. After stirring for 75 min, the mixture was poured into ice-H$_2$O (60 ml), acidified with 0.1 N HCl, and extracted with CHCl$_3$. Evaporation of the CHCl$_3$ extract gave a colorless crystalline material which was purified by recrystallization from aqueous Me$_2$CO to afford 14 as colorless needles (70 mg): mp 191–193°; $[\alpha]^{23}$D $-48.6°$ ($c = 0.62$, CHCl$_3$); ir ν max (KBr) 3300, 1720, 1660, 1600, 1580 cm^{-1}; uv λ max (MeOH) (log ϵ) 229 (4.51), 267 (3.42), 272 (3.44), 278 sh (3.35) nm; ^{1}H nmr (CDCl$_3$) δ 1.16 (3H, s, Me-17), 1.35 (3H, s, Me-19), 1.51 (3H, s, Me-20), 5.31 (1H, s, H-6), 7.45 (2H, t, $J = 7.3$ Hz, H-3', -5'), 7.56 (1H, t, $J = 7.3$ Hz, H-4), 8.02 (2H, d, $J = 7.3$ Hz, H-2', -6'); hrms *m/z* [M]$^+$ 453.2525 (calcd for C$_{27}$H$_{35}$NO$_5$, 453.2513). *Anal.* calcd for C$_{27}$H$_{35}$NO$_5$, C 71.48, H 7.78, N 3.09; found C 71.20, H 7.97, N 3.13.

PREPARATION OF METHYL ESTER 15.—Compound 15 was obtained by treating 14 with ethereal CH$_2$N$_2$ in the usual manner. Colorless amorphous powder: $[\alpha]^{23}$D $-29.9°$ ($c = 0.42$, CHCl$_3$); ir ν max (CHCl$_3$) 3300, 1715, 1600, 1280 cm^{-1}; uv λ max (MeOH) (log ϵ) 229 (4.19), 267 (3.13), 272 (3.17), 278 sh (3.08) nm; ^{1}H nmr (CDCl$_3$) δ 1.17 (3H, s, Me-17), 1.34 (3H, s, Me-19), 1.50 (3H, s, Me-20), 3.67 (3H, s, OMe), 5.19 (1H, s, H-6), 7.45 (2H, t, $J = 7.3$ Hz, H-3', -5'), 7.57 (1H, t, $J = 7.3$ Hz, H-4'), 8.02 (2H, d, $J = 7.3$ Hz, H-2', -6'); hrms *m/z* [M]$^+$ 467.2718 (calcd for C$_{28}$H$_{37}$NO$_5$, 467.2670).

PREPARATION OF LACTONE 16.—To a solution of 7 (42 mg) in dry C$_6$H$_6$ (0.5 ml) was added trifluoroacetic anhydride (50 μl), and the mixture was left for 10 min. After dilution with H$_2$O, the reaction product was extracted with C$_6$H$_6$. The C$_6$H$_6$ extract was subjected to a Si gel cc. Elution with CHCl$_3$ gave 16 as colorless needles (29 mg): mp 203–204°; $[\alpha]^{23}$D $+85.3°$ ($c = 0.53$, CHCl$_3$); ir ν max (CHCl$_3$) 1770, 1710 cm^{-1}; ^{1}H nmr (CDCl$_3$) δ 1.11 (3H, s, Me-17), 1.29 (3H, s, Me-19), 1.48 (3H, s, Me-20), 4.71 (1H, m, H-6); hrms *m/z* [M]$^+$ 316.2043 (calcd for C$_{20}$H$_{28}$O$_3$, 316.2037). *Anal.* calcd for C$_{20}$H$_{28}$O$_3$, C 75.90, H 8.92; found C 75.91, H 8.83.

PREPARATION OF TRIACETATE 19.—Compound 18 (25 mg) was acetylated with 4-dimethyl-

aminopyridine (3 mg), Ac_2O (0.2 ml), and pyridine (0.2 ml) at room temperature to give triacetate **19** (23 mg) as colorless needles: mp 155–156°; $[\alpha]^{23}D$ −60.1° ($c = 0.56$, $CHCl_3$); ir ν max ($CHCl_3$) 1725, 1720, 1250 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.94 (3H, s, Me-17), 1.01 (3H, s, Me-19), 1.30 (3H, s, Me-20), 2.02 (3H, s, OAc), 2.04 (6H, s, 2 × OAc), 3.69 and 3.86 (each 1H, d, $J = 11.2$ Hz, H-18), 4.63 (1H, dd, $J = 10.7$, 5.9 Hz, H-13), 5.25 (1H, d, $J = 2.4$ Hz, H-6); hrms m/z [M]$^+$ 448.2870 (calcd for $C_{26}H_{40}O_6$, 448.2823). *Anal.* calcd for $C_{26}H_{40}O_6$, C 69.60, H 8.99; found C 69.34, H 8.97.

PREPARATION OF **29** AND **30**.—Compounds **27** and **28** were acetylated with Ac_2O/pyridine in the usual manner to yield **29** and **30**, respectively.

Compound **29** was obtained as colorless needles: mp 204–207°; $[\alpha]^{23}D$ −47.4° ($c = 0.24$, $CHCl_3$); ir ν max ($CHCl_3$) 1715, 1600 cm^{-1}; uv λ max (MeOH) (log ϵ) 229 (4.35), 267 (3.24), 272 (3.31), 278 sh (3.28) nm; ^{1}H nmr ($CDCl_3$) δ 0.93 (3H, s, Me-17), 1.36 (3H, s, Me-19), 1.55 (3H, s, Me-20), 2.03 (3H, s, OAc), 3.66 (3H, s, OMe), 4.62 (1H, d, $J = 3.4$ Hz, H-13), 5.14 (1H, d, $J = 2.4$ Hz, H-6), 7.48 (2H, t, $J = 7.3$ Hz, H-3', -5'), 7.59 (1H, t, $J = 7.3$ Hz, H-4'), 8.07 (2H, d, $J = 7.3$ Hz, H-2', -6'); hrms m/z [M]$^+$ 438.2500 (calcd for $C_{27}H_{34}O_5$, 438.2404). *Anal.* calcd for $C_{27}H_{36}O_5 \cdot C_3H_6O$, C 72.5, H 8.12; found C 72.40, H 8.36.

Compound **30** was obtained as a colorless amorphous powder: $[\alpha]^{23}D$ −60.3° ($c = 0.46$, $CHCl_3$); ir ν max ($CHCl_3$) 1715, 1600 cm^{-1}; uv λ max (MeOH) (log ϵ) 229 (4.37), 267 (3.22), 272 (3.26), 278 sh (3.18) nm; ^{1}H-nmr ($CDCl_3$) δ 0.94 (3H, s, Me-17), 1.33 (3H, s, Me-19), 1.57 (3H, s, Me-20), 2.01 (3H, s, OAc), 3.67 (3H, s, OMe), 4.61 (1H, dd, $J = 11.2$, 5.7 Hz, H-13), 5.15 (1H, d, $J = 1.9$ Hz, H-6), 7.46 (2H, t, $J = 7.3$ Hz, H-3', -5'), 7.55 (1H, t, $J = 7.3$ Hz, H-4'), 8.02 (2H, d, $J = 7.3$ Hz, H-2', -6'); hrms m/z [M]$^+$ 438.2378 (calcd for $C_{27}H_{34}O_5$, 438.2404). *Anal.* calcd for $C_{27}H_{34}O_5 \cdot C_3H_6O$, C 72.54, H 8.12; found C 72.27, H 8.30.

PREPARATION OF GASTRIC VESICLES.—Gastric vesicles enriched in H^+, K^+-ATPase were prepared from mucosa in the fundic region of hog stomachs by differential and density gradient centrifugation as described elsewhere (21). The mucosa were homogenized in homogenizing buffer containing 250 mM sucrose, 1 mM EGTA and 5 mM Tris/HCl (pH 7.4). The microsomal fraction was centrifuged through 250 mM sucrose layered on a 7% (w/v) Ficoll plus 250 mM sucrose step gradient. The interface fraction, comprising intact gastric vesicles, was diluted with 10 volumes of distilled H_2O, followed by immediate freezing in liquid N_2. Then the vesicles were lyophilized and resuspended with the original volume of distilled H_2O. Vesicle preparations were stored at −80° and used within 2 or 3 weeks.

ENZYME ASSAY.—H^+, K^+-ATPase activity was measured in a 1 ml solution containing gastric vesicles (10 μg protein), 3 mM $MgSO_4$, 3 mM ATP, and 40 mM Tris/HCl buffer (pH 7.4) with or without 15 mM KCl as described elsewhere (19). The ATPase activity was expressed as μmol of inorganic phosphate per mg of protein per h. H^+, K^+-ATPase activity was calculated as the difference between ($Mg^{2+} + K^+$)-dependent activity and Mg^{2+}-dependent activity.

EXAMINATION OF H_2O SOLUBILITY.—EtOH solutions of test compounds (5–10 mM) were diluted with 40 mM Tris/HCl buffer (pH 7.4), and the absorbance of each solution (1–50 μM) at 550 nm was determined.

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