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Studies on the Absorption, Distribution, Excretion and Metabolism of Ginseng Saponins. IV.[1] Decomposition of Ginsenoside-Rg_1 and -Rb_1 in the Digestive Tract of Rats

TSUTOMU ODANI,* HISAYUKI TANIZAWA, and YOSHIO TAKINO

Shizuoka College of Pharmacy, 2–2–1, Oshika, Shizuoka 422, Japan

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The decomposition of ginsenoside-Rg_1 and ginsenoside-Rb_1 in the rat stomach and large intestine after oral administration was investigated. In the stomach, a part of ginsenoside-Rg_1 was decomposed and six decomposition products were observed on a reversed phase thin-layer chromatogram (TLC). These six compounds were identical with those which were obtained on hydrolysis of ginsenoside-Rg_1 under mild acidic conditions (with 0.1 N HCl, at 37 °C). On the other hand, an unidentified deomposition product of ginsenoside-Rb_1 was observed on the TLC of the stomach sample after the oral administration of Rb_1 to rats. The product was different from the decomposition product formed by the hydrolysis of Rb_1 under mild acidic conditions. In the large intestine, Rg_1 was decomposed to ginsenoside-Rh_1 and ginsenoside-F_1 by tetracycline-susceptible bacteria and tetracycline-resistant bacteria, respectively. Ginsenoside-Rb_1 was decomposed to ginsenoside-Rd and two unidentified products by enteric enzyme and tetracycline-resistant bacteria, respectively.

Keywords——ginsenoside-Rg_1; ginsenoside-Rb_1; ginsenoside-Rh_1; ginsenoside-Rd; ginsenoside-F_1; ginsenoside prosapogenin; decomposition; ^{13}C-NMR; reversed phase TLC; rat digestive tract

Previously, we[1] have reported on the absorption, distribution and excretion of ginsenoside-Rg_1 (Rg_1) and ginsenoside-Rb_1 (Rb_1) after oral administration of Rg_1 or Rb_1 to rats. We also showed that both Rg_1 and Rb_1 were partially decomposed in the stomach and large intestine of rats after oral administration. On the other hand, Han *et al.*[2] reported on the decomposition of Rg_1 and Rb_1 by mild acid (0.1 N HCl solution), assuming that these ginsenosides were possibly decomposed by gastric juice in the stomach. However, there is no report on the decomposition of Rg_1 or Rb_1 in the digestive tract after oral administration to rats. In this paper, therefore, the decomposition of Rg_1 and Rb_1 in the digestive tract of rats after oral administration was investigated.

Experimental

Materials——Rg_1 and Rb_1 used were those described previously.[1] Crude hesperidinase was kindly supplied by Tanabe Pharm. Co., Ltd. Acromycin® V (tetracycline hydrochloride) was purchased from Takeda Chemical Industries Co., Ltd. Other reagents were the same as described in our previous paper.[1] Experimental animals used were male Sprague–Dawely (JCL: SD. SPF) rats weighing 180—200 g. The rats were deprived of food but given free access to water for 18 h prior to the experiments.

^{13}C-Nuclear Magnetic Resonance (^{13}C-NMR)——^{13}C-NMR spectra were measured with a Varian FT-80 spectrometer.

Thin-Layer Chromatography (TLC)——Normal phase TLC was performed on Silica gel 60 pre-coated plates, using the following solvent systems as developing solvents: (A), 1-butanol (BuOH)–acetic acid–H_2O (4 : 1 : 5, upper phase); (B), $CHCl_3$–BuOH–methanol (MeOH)–H_2O (20 : 40 : 15 : 20, lower phase); (C), $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase). Reversed phase TLC was performed on Silica gel 60 silanized plates by using 60% MeOH as the

developing solvent. Detection was achieved by spraying 8% vanillin in MeOH–72% H_2SO_4 (1 : 5, v/v) followed by heating (140 °C, 3—4 min).

A. Chemical and Enzymatic Decomposition of Rg_1 and Rb_1——1) Hydrolysis of Rg_1 and Rb_1 with 0.1 N HCl Solution: Five mg of Rg_1 or Rb_1 was dissolved in 1 ml of 0.1 N HCl, and incubated at 37 °C for 1 h. Then, the reaction mixture was treated with a SEP-PAK® cartridge according to the previous paper,[1b] and the hydrolysis products were obtained.

2) Isolation of Hydrolysis Products (II_1, II_2) of Rg_1: Rg_1 (500 mg) was dissolved in 10 ml of 0.1 N HCl, and incubated at 37 °C for 15 min. The reaction mixture was centrifuged at 3500 rpm for 10 min and the supernatant was incubated at 37 °C for 15 min again. These processes were repeated until no further precipitation occurred. The resulting precipitates (372 mg) were combined. The precipitates in 60% MeOH were chromatographed on a C_{18} silica gel column (1.5 × 29 cm) with 60% MeOH. Rechromatography on the C_{18} silica gel column, eluting with same solvent, gave two compounds II_1 (yield 62.7 mg) and II_2 (yield 96.5 mg).

3) Isolation of Hydrolysis Products (III_1, III_2) of Rg_1: II_1 (90 mg) was suspended in 20 ml of 0.1 N HCl, and incubated at 37 °C for 96 h. The incubation mixture was treated with SEP-PAK® to obtain a mixture (83 mg) of III_1 and III_2. The mixture was chromatographed as described above to give two compounds, III_1 (yield 27 mg) and III_2 (yield 25.3 mg).

4) Isolation of Ginsenoside-F_1 Formed from Rg_1 by Crude Hesperidinase:[3] Rg_1 (300 mg) was disssolved in 10 ml of 0.2 M Na_2HPO_4–0.1 M citric acid buffer (pH 4.0), and crude hesperidinase (100 mg) was added. After incubation at 37 °C for 10 min, the mixture was centrifuged at 3500 rpm for 7 min, to obtain white precipitates. The supernatant was reincubated in the same manner as above, and these processes were repeated until no further precipitation occurred. The combined precipitate was desalted by Servachrome® XAD-2 resin column chromatography according to our previous paper,[1a] and the MeOH eluate was evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (9 : 1) to afford the main product (153 mg), which was identified as ginsenoside-F_1 by comparison of ^{13}C-NMR spectral data with the reported values.[4]

B. Biological Decomposition of Rg_1 and Rb_1——1) Oral Administration: After oral administration of Rg_1 and Rb_1 (100 mg/kg, 2% aqueous solution) separately to rats, the stomach and large intestine were isolated and treated according to the procedure described in the previous paper.[1]

2) Decomposition of Rg_1 and Rb_1 in the Cecum: a) Rats were anesthetized with pentobarbital sodium (35 mg/kg, *i.p.*). After laparotomy, the ileocecal part and the end of the cecum were separately ligated. One percent Rg_1 or Rb_1 solution in 0.9% saline was injected into the cecume at a dose of 5 mg/kg.

b) Tetracycline hydrochloride (100 mg/kg, *p.o.*) was administered to rats at 12 h intervals for 3 d. At 3 h after the last administration, the rats were treated as in a).

3) Decomposition of Rg_1 and Rb_1 by Rat Cecum Content (*in Vitro*): The whole cecal content of a normal rat was suspended in 6 ml of saline, and the suspension was divided into 3 parts. Two parts were centrifuged at 11000 rpm for 30 min and passed through a membrane filter (0.22 μm), and one of the filtrates was heated at 100 °C for 30 min. One mg of Rg_1 or Rb_1 was added to each filtrate or the intact part and incubated at 37 °C for 2 h (Rb_1) or 4 h (Rg_1). The decomposition products of Rg_1 or Rb_1 were investigated in a manner similar to that used previously.[1]

4) Isolation of Decomposition Product of Rb_1 in Cecal Content of Normal Rats: The whole cecal content of a normal rat was suspended in 4 ml of 0.9% saline solution containing 22.5 mg of Rb_1, and was incubated at 37 °C for 2.5 h. To this, 25 ml of MeOH was added, and supernatant was obtained by centrifugation (3500 rpm, 10 min). Further, these processes were repeated 10 times. The combined supernatant was evaporated to dryness under reduced pressure. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase) followed by C_{18} silica gel column chromatography using 50% MeOH to afford a white powder (78.6 mg) which was identified as ginsenoside-Rd (VII) from its TLC behavior and IR and ^{13}C-NMR spectra.

Results and Discussion

Decomposition of Rg_1 in Rat Stomach

As shown in Fig. 1a, three decomposition products (I, II, III) were observed by the TLC method in a stomach content sample taken 30 min after the administration of Rg_1 (100 mg/kg, *p.o.*). These three products were identified as Rg_1-prosapogenins, I, II and III, respectively. Han *et al.*[2] have reported that these compounds are formed from Rg_1 by decomposition under mild acidic conditions. However, each of the three products (I, II, and III) was further separated into two compounds I_1 and I_2, II_1 and II_2, and III_1 and III_2, respectively, by reversed phase TLC (Fig. 1b). All the ^{13}C-NMR signals of II_1 were superimposable on those of ginsenoside-Rh_1 (Rh_1). The ^{13}C-NMR spectrum of II_2 was different from that of II_1 (Rh_1) in the signals arising from 17-C, 21-C and 22-C (Table I). Asakawa *et al.*[5] reported that the 20-C

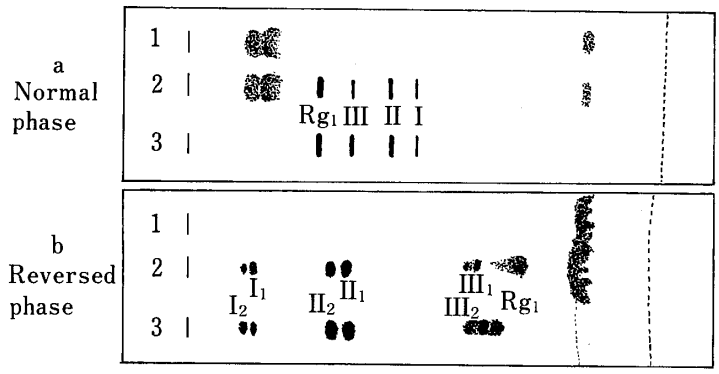
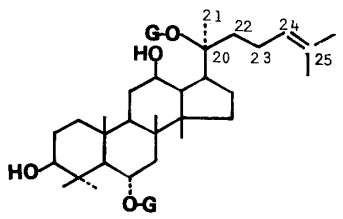
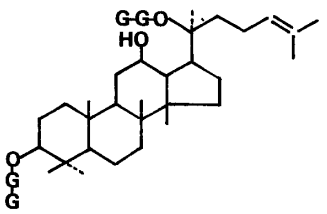


Fig. 1. Thin-Layer Chromatograms of Decomposition Products of Ginsenoside-Rg_1 in Rat Stomach or in 0.1 N HCl Solution

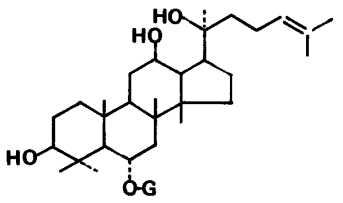
Developing solvents: a, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase); b, 60% MeOH. Plates: a, Merck precoated silica gel 60; b, Merck precoated silica gel 60 silanized. Detecting reagent: 8% vanillin–MeOH solution/72% H_2SO_4 (1 : 5), with heating at 140 °C for 3 min. 1, normal rat; 2, Rg_1 (100 mg/kg, *p.o.*)-administered rat (30 min after treatment); 3, 0.1 N HCl solution (37 °C, 1 h).



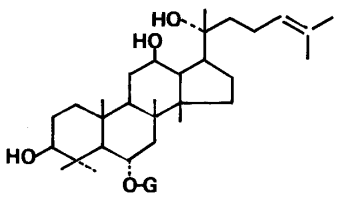
ginsenoside Rg_1



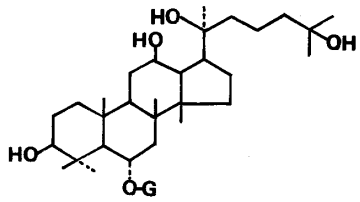
ginsenoside Rb_1



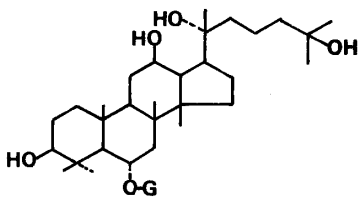
II_1 (Rh_1)



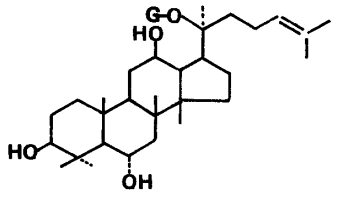
II_2



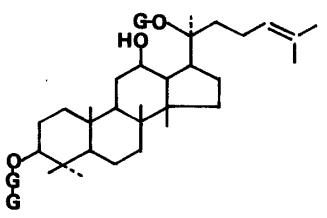
III_1



III_2



ginsenoside F_1



ginsenoside Rd

G: β-D-glucose

Chart 1

TABLE I. ^{13}C-NMR Chemical Shifts of Hydrolysis Products of Rg_1

Carbon No.	Compound			
	II_1	II_2	III_1	III_2
Aglycone moieties				
1	39.4	39.4	39.4	39.4
2	27.7	27.7	27.9	27.9
3	78.7[a]	78.7[a]	78.6[a]	78.6[a]
4	40.2	40.3	40.3	40.3
5	61.4	61.4	61.4	61.4
6	77.9[a]	77.9[a]	78.1[a]	78.0[a]
7	45.1	45.2	45.7	45.5
8	41.1	41.1	41.1	41.1
9	50.2	50.2	50.2	50.2
10	39.6	39.6	39.7	39.7
11	31.9[b]	32.0[b]	32.1[b]	32.2[b]
12	71.0	70.9	71.0	70.9
13	48.1	48.8	48.2	49.0
14	51.6	51.7	51.6	51.7
15	31.2[b]	31.3[b]	31.3[b]	31.3[b]
16	26.9	26.6	26.9	26.7
17	54.6	50.5	54.7	50.8
18	17.6[c]	17.6[c]	17.6[c]	17.7[c]
19	17.3[c]	17.4[c]	17.4[c]	17.4[c]
20	73.1	73.2	73.3	73.4
21	26.9	22.6	26.9	22.7
22	35.8	43.1	36.4	44.0
23	22.9	22.6	19.1	18.7
24	126.2	126.0	45.2	45.2
25	130.8	130.8	69.7	69.8
26	17.7[c]	17.7[c]	29.8	29.9
27	25.8	25.8	30.1	30.1
28	31.6	31.6	31.7	31.7
29	16.8[c]	17.1[c]	16.8[c]	17.1[c]
30	16.3[c]	16.3[c]	16.4[c]	16.3[c]
Sugar moieties				
1′	105.8	105.8	105.9	105.9
2′	75.4	75.4	75.4	75.4
3′	80.0[a]	80.0[a]	80.0[a]	80.0[a]
4′	71.8	71.8	71.8	71.9
5′	79.4[a]	79.4[a]	79.5[a]	79.5[a]
6′	63.0	63.0	63.1	63.1

δ ppm from internal TMS in C_5D_5N.
a—*c*) These assignments may be reversed in each column.

epimeric pair of a dammarane type triterpene could not be distinguished in terms of TLC behavior, optical rotation, infrared (IR), mass spectrum (MS) or ^{1}H-NMR spectrum. They claimed that ^{13}C-NMR was the most suitable method for that purpose, since obvious differences in ^{13}C-NMR chemical shifts of 13-C, 16-C, 17-C, 20-C, 21-C and 22-C were observed in 20-C epimeric pairs. Therefore, as shown in Table I, II_2 can be defined as the 20-C(*R*) epimer of Rh_1 (20-C(*S*) epimer, Chart 1).

All the ^{13}C-NMR signals of III_1 were superimposable on those of the C-25,26-hydrated derivative of Rh_1,[2] but the ^{13}C-NMR chemical shifts of III_2 at 17-C, 21-C and 22-C were different from those of III_1. The relationship between III_1 and III_2 was the same as that

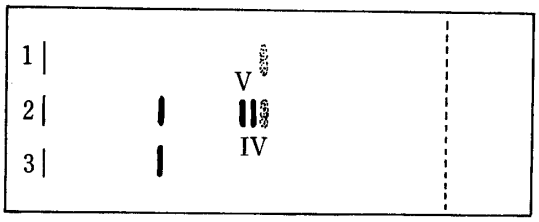


Fig. 2. Thin-Layer Chromatogram of Decomposition Products of Ginsenoside-Rg_1 in Rat Large Intestine

Developing solvent: $CHCl_3$–MeOH–H_2O (65:35:10, lower phase). 1, normal rat; 2, Rg_1 (100 mg/kg, *p.o.*)-administered rat (6 h after treatment); 3, standard Rg_1.

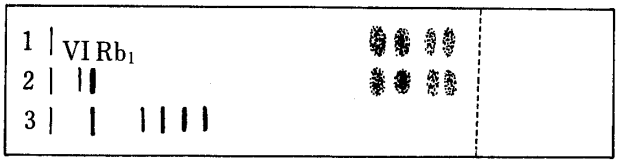


Fig. 3. Thin-Layer Chromatogram of Decomposition Products of Ginsenoside-Rb_1 in Rat Stomach or in 0.1 N HCl Solution

Developing solvent: $CHCl_3$–MeOH–H_2O (65:35:10, lower phase). 1, normal rat; 2, Rb_1 (100 mg/kg, *p.o.*)-administered rat (30 min after treatment); 3, 0.1 N HCl solution (37 °C, 1 h).

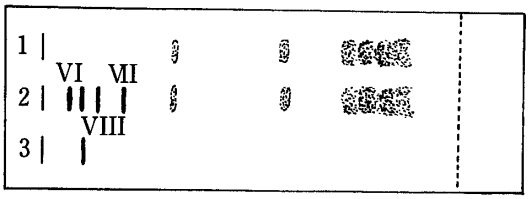


Fig. 4. Thin-Layer Chromatogram of Decomposition Products of Ginsenoside-Rb_1 in Rat Large Intestine

Developing solvent: $CHCl_3$–MeOH–H_2O (65:35:10, lower phase). 1, normal rat; 2, Rb_1 (100 mg/kg, *p.o.*)-administered rat (4 h after treatment); 3, standard Rb_1.

between II_1 and II_2. Compound III_2 was inferred to be the C-25,26-hydrated derivative of the 20-C(*R*) epimer of Rh_1.

Compounds I_1 and I_2 were also isolated by C_{18} silica gel column chromatography, but their structures could not be determined, because these compounds were unstable as pointed out by Han *et al.*[2] In the incubation of Rg_1 with 0.1 N HCl at 37 °C, variation in the formation of products was observed. Compounds I and II were initially produced, and later III was formed. It was considered that I was the compound formed by elimination of the 20-C-glycosyloxy moiety or 20-C-hydroxy group in Rg_1, corresponding to Δ^{20}-prosapogenin reported by Kaku *et al.*[6] in the hydration of 20(*S*)-protopanaxadiol group saponins and closely related to II and III. From these results, it has become apparent that Rg_1 was decomposed under mild acidic conditions or in the rat stomach to yield six prosapogenins.

Decomposition Products of Rg_1 in Rat Large Intestine

A thin-layer chromatogram of decomposition products (IV, V) of Rg_1 in the large intestine 6 h after oral administration of Rg_1 (100 mg/kg) is shown in Fig. 2. The products IV and V were identified as ginsenoside-F_1 and Rh_1, respectively, by normal phase TLC and reversed phase TLC.

Decomposition Products of Rb_1 in Rat Stomach

A thin-layer chromatogram of decomposition products of Rb_1 in the stomach and with 0.1 N HCl at 37 °C is shown in Fig. 3. A decomposition product (VI) of Rb_1 in the stomach was not found in the sample obtained by the treatment of Rb_1 with 0.1 N HCl. The *Rf* value of VI on TLC (solvent C) was lower than that of Rb_1 or the decomposition products with 0.1 N HCl. Therefore, it is unlikely that VI is a hydrolysis product formed by elimination of *O*-glycosyl moieties. The amount of VI was too small to permit determination of its chemical structure. It appears that Rb_1 was decomposed to VI only in the rat stomach.

The Decomposition Products of Rb_1 in the Large Intestine

A thin-layer chromatogram of decomposition products (VI, VII, VIII) of Rb_1 in the large intestine of a rat 4 h after oral administration of Rb_1 (100 mg/kg) is shown in Fig. 4. The same

TABLE II. Comparison of Rg_1 and Rb_1 Decomposition Rat Cecum (*in Vivo*) and 0.9% Saline Suspension of Rat Cecal Content (*in Vitro*)

Treatment	Products				
	Rg_1		Rb_1		
	IV (F_1)	V (Rh_1)	VI	VII (Rd)	VIII
In vivo					
Normal rat	+	+	+	++	+
Tetracycline-[a] treated rat	+	−	+	++	+
In vitro[b]					
Normal rat	+	+	+	++	+
Sterile filtrate of normal rat	−	−	−	++	−
Heated sterile filtrate	−	−	−	−	−

a) Tetracycline treatment: 100 mg/kg × 2/d × 2 d.
b) Incubated at 37 °C for 3 h (Rg_1) or 1 h (Rb_1).

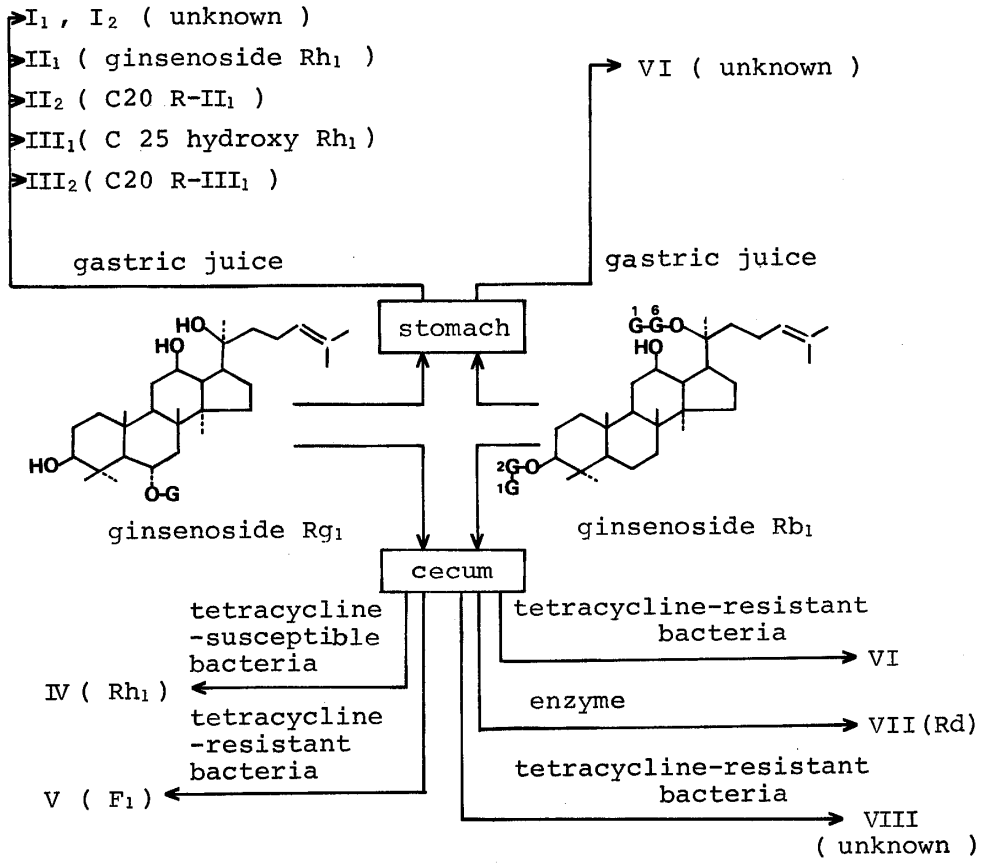


Chart 2. Decomposition of Ginsenoside-Rg_1 and -Rb_1 in the Stomach and Cecum of Rats

products were also found in the sample obtained from the ligated cecum into which Rb_1 had been directly injected and allowed to stand for 1.5 h. Therefore, VI was formed in both the large intestine and stomach of rats. The structures of VI and VIII are under study. However, VII, a main decomposition product of Rb_1 in the large intestine, was identified as ginsenoside-Rd by TLC, and by comparison of IR and ^{13}C-NMR data.[7]

Mode of Decomposition of Rg_1 and Rb_1 in Rat Cecum

As shown in Table II, the decomposition products which were formed by the incubation of Rg_1 and Rb_1 with the cecal content of a normal rat were identical with those obtained from the large intestine or ligated cecum after oral administration or direct injection of Rg_1 and Rb_1. However, V (Rh_1) was not formed from Rg_1 in the cecum of a rat treated with tetracycline hydrochloride. The products IV, V, VI and VIII were also not formed from Rg_1 and Rb_1 with the sterile filtrate of cecal content suspended in 0.9% saline solution. In addition, VII was not produced with sterile filtrate heated at 100 °C for 30 min. From these results, it seems that V was produced by tetracycline-susceptible bacteria and IV, VI and VIII, and VII were produced by tetracycline-resistant bacteria and an enteric enzyme, respectively.

The patterns of decomposition of Rg_1 and Rb_1 in rat stomach and large intestine are summarized in Chart 2. The chemical structures of Rb_1, Rg_1 and their decomposition products that were identified are shown in Chart 1. On the other hand, we have already reported in the previous paper[1] that these decomposition products could not be found by the TLC method. Thus, these products are suggested to be poorly absorbed from the rat digestive tract, and might not play a major role in the pharmacological activities of Rg_1 or Rb_1.

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