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METABOLISM OF GLYCYRRHETIC ACID BY RAT LIVER MICROSOMES—II

22α- AND 24-HYDROXYLATION

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Abstract—18β-Glycyrrhetic acid (GA, an aglycone of glycyrrhizin) is converted to 3-oxo-18β-glycyrrhetic acid (3-oxoGA) in the presence of $NADP^+$ by rat liver homogenates, but GA was converted in the presence of NADPH to two other metabolites showing lower R_f values on thin-layer chromatography (TLC) than those of GA and 3-oxoGA by postmitochondrial supernatant of rat liver. The GA-metabolizing activity in the presence of NADPH was localized in microsomes, similar to localization of GA-oxidizing activity to 3-oxoGA. The GA-metabolizing activity required NADPH as a cofactor and O_2 for full activity and was inhibited with CO, suggesting the hydroxylation reaction of GA by cytochrome P450. Two metabolites (I and II, lower and higher R_f values on TLC, respectively) were purified on preparative TLC. Mass spectral (MS) analyses of II and methyl ester of acetylated I indicated the formation of monohydroxylated metabolites. On the basis of ^3H- and ^{13}C-NMR assignments I and II were identified to be 22α- and 24-hydroxy-18β-glycyrrhetic acids, respectively. 3-OxoGA and 3-epi-18β-glycyrrhetic acid (3-epiGA) seem to be also hydroxylated at C-22 and C-24. A metabolite of 3-oxoGA showing a lower R_f value was also identified as 22α-hydroxy-3-oxo-18β-glycyrrhetic acid by MS and ^3H- and ^{13}C-NMR spectral analyses. In 22α-hydroxylation the best substrate was 3-oxoGA, followed by GA and 3-epiGA. On the other hand, for 24-hydroxylation the best substrate was GA, then 3-oxoGA, and 3-epiGA in order. However, 18α-glycyrrhetic acid (18α-GA) was a poor substrate for both 22α- and 24-hydroxylation.

Humans ingest glycyrrhizin (GL, glycyrrhizic acid‖) orally as a sweetener or as a main constituent of liquorice extract (*Glycyrrhiza glabra* L.) which is widely used in oriental medicine. However, GL is not detected in the sera of human subjects after oral administration of GL, but 18β-glycyrrhetic acid (GA, an aglycone of GL) is found [1]. Even if GL is injected intravenously, GA is also detected in their sera [1]. On the other hand, GL is hydrolysed to GA and then metabolized to 3-epi-18β-glycyrrhetic acid (3-epiGA) via 3-oxo-18β-glycyrrhetic acid (3-oxoGA) by human intestinal bacteria [2–4]. Accordingly, when GL is administered orally, GA, not GL, seems to be absorbed after the hydrolysis of GL to GA in intestine. Carbenoxolone, 3-*O*-β-carboxypropionyl-GA, orally administered is also hydrolysed to GA only in the gut of rodents before absorption [5]. Although in the human carbenoxolone is absorbed mostly unchanged after oral administration, it is excreted in the bile as the glucuronide [6]. Moreover, GA is the effective anti-inflammatory agent [7, 8]. Thus, it is important to clarify the metabolism of GA in animal tissues. However, studies on GA-metabolizing capacities of animal tissues are scarce, except those on glucuronidation and sulfation of GA in rat *in vivo* followed by biliary excretion [5], though hydroxylation of GA by bacteria such as *Streptomyces* [9] and *Chaina antibiotica* [10] has been reported.

Recently, we discovered the reversible conversion of GA to 3-oxoGA by rat liver microsomes and partially purified GA dehydrogenase from the microsomes [11]. In the present paper we report that GA, 3-oxoGA and 3-epiGA are hydroxylated by rat liver microsomes, and that these hydroxylation products are 22α- and 24-hydroxyl derivatives.

MATERIALS AND METHODS

Apparatus

^1H- and ^{13}C-Nuclear magnetic resonance (NMR) spectra were measured with a JEOL FX-90Q (^{13}C, 22.5 MHz), a JEOL GX-270 (^1H, 270 MHz) and a JEOL GX-400 (^1H, 400 MHz; ^{13}C, 100 MHz) spectrometers using tetramethylsilane as an internal standard. Mass spectra (MS) were measured with a JEOL JMS DX-300 mass spectrometer at an ionization voltage of 70 eV.

Chemicals

GA was purchased from Nacalai Tesque, Inc. (Kyoto, Japan) and purified by repeated crystallization. 3-EpiGA and 3-oxoGA were prepared as described in the previous paper [3]. 18α-Glycyrrhetic acid (18α-GA) was given by Maruzen Seiyaku Co.

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‖ Abbreviations used: GA, 18β-glycyrrhetic acid; 3-oxoGA, 3-oxo-18β-glycerrhetic acid; 3-epiGA, 3-epi-18β-glycyrrhetic acid; 18α-GA, 18α-glycyrrhetic acid; GL, glycyrrhizin; MS, mass spectra.

(Onomichi, Japan). Glycyrrhetic acid mono-β-D-glucuronide was donated by Dr M. Kanaoka of the Research Institute for Wakan-Yaku (Traditional Sino-Japanese Medicines) of our University. Above compounds were chromatographically pure by TLC and HPLC, and their purity was certified by ^{3}H- and ^{13}C-NMR. GL monoammonium salt was purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). NADPH, NADH, $NADP^+$ and NAD^+ were products of Oriental Yeast Co., Ltd (Tokyo, Japan). All other reagents were of the best commercial quality available.

Preparation of subcellular fractions and microsomes

Liver homogenates in 0.25 M sucrose were separated into nuclear, mitochondrial, lysosomal, microsomal and soluble fractions with minor modifications of the methods by Imai *et al.* [12]. Liver microsomes were prepared from Wistar rats as described previously [13] except that 0.15 M KCl was used for the homogenation of perfused livers instead of 0.25 M sucrose.

Thin-layer chromatography (TLC)

TLC for GA, 3-epiGA, 3-oxoGA, 18α-GA and the hydroxylated derivatives was performed on silica gel plates (Merck, silica gel 60 F-254, layer thickness 0.25 mm) with a solvent system of chloroform/petroleum ether (b.p. range, 30–60%)/acetic acid (5:5:1 by vol.). TLC for GL and GA monoglucuronide was performed on the same plates with a solvent system of acetic acid/*n*-butanol/1,2-dichloroethane/H_2O (4:1:4:1 by vol.). The quantity was analysed with a TLC scanner as described in the previous paper [4].

Assay

The enzyme activities for hydroxylation of GA, 3-epiGA, 3-oxoGA, 18α-GA, GL and GA monoglucuronide were measured as follows.

The assay mixture contained 50 nmol of substrate, 0.5 μmol of NADPH, 100–500 μg of microsomal protein and 0.1 M potassium phosphate buffer (pH 7.2) in a final volume of 0.5 mL. The mixture was incubated at 37° for 10–30 min, and the reaction was stopped by the addition of 0.1 N HCl. It was then extracted twice with 3 mL of ethyl acetate. After evaporating the ethyl acetate phase, the residue was analysed by TLC as described above.

Protein was determined by the method of Lowry *et al.* [14].

Isolation of two hydroxylation products (I and II) of GA

Liver microsomes of female rats (36 weeks old) were used for the production of I (showing the lower R_f value than that of II on TLC, Fig. 1). The reaction mixture contained 340 mg of microsomal protein, 41 μmol of GA and 300 μmol of NADPH in 200 mL of 0.1 M potassium phosphate buffer (pH 7.2). On the other hand, liver microsomes of male rats (8 weeks old) treated with phenobarbital (80 mg/kg, i.p., 4 days) were used for the production of II. The reaction mixture contained 820 mg of microsomal protein, 60 μmol of GA and 300 μmol of NADPH in 300 mL of 50 mM potassium phosphate buffer

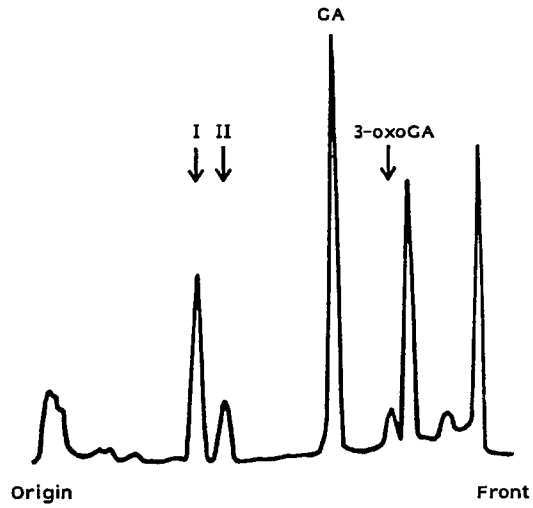

Fig. 1. TLC profile of GA-metabolites. GA was incubated with postmitochondrial supernatant of male rat liver in the presence of NADPH in 0.1 M potassium phosphate buffer (pH 7.2). After incubation for 30 min at 37° the extract from the reaction mixture was analysed by TLC as described in Materials and Methods. I, II and 3-oxoGA (shown by arrows) were metabolites of GA, and the other peaks were due to contaminants present in the postmitochondrial supernatant.

(pH 7.2). After incubation at 37° for 1 hr, the reaction was stopped by the addition of 1 N HCl. Then, the products were extracted twice with an equal volume of ethyl acetate, and isolated by preparative TCL after evaporating the ethyl acetate. In the former case one major product (I) was obtained in addition to a trace amount of II, and in the latter case a large amount of II and a small amount of I were obtained.

22α-Hydroxyglycyrrhetic acid (I). ^{1}H-NMR (270 MHz, CD_3OD): δ0.84(3H, s, Me), 0.97(3H, s, Me), 1.03(3H, s, Me), 1.16(6H, s, Me × 2), 1.17(3H, s, Me), 1.47(3H, s, Me), 3.23(1H, dd, J = 11.7, 4.6 Hz, H-3), 3.55(1H, dd, J = 12.1, 4.5 Hz, H-22), 5.73(1H, s, H-12). MS *m/z* (rel. int.): 468(M^+-H_2O, 17), 450(42), 435(26), 407(22), 301(100), 260(52).

3-O-Acetyl-22α-hydroxyglycyrrhetic acid (Ia). I was treated with acetic anhydride in pyridine to give an acetyl derivative. MS *m/z* (rel. int.): 509(M^+-H_2O, 6), 449(M^+-AcOH-H_2O, 56), 434(40), 406(72), 300(100). ^{1}H-NMR (270 MHz, CD_3OD): δ0.85(3H, s, Me), 0.897(3H, s, Me), 0.904(3H, s, Me), 1.12(3H, s, Me), 1.14(3H, s, Me), 1.16(3H, s, Me), 1.46(3H, s, Me), 2.03(3H, s, AcO-3), 3.55(1H, dd, H-22), 4.48(1H, dd, J = 11.4, 5.7 Hz, H-3), 5.72(1H, s, H-12).

3-O-Acetyl-22α-hydroxyglycyrrhetic acid methyl ester (Ib). Ia was methylated with diazomethane to give a monomethyl ester. MS *m/z* (rel. int.): 542(M^+, 42), 482(M^+-AcOH, 17), 464(M^+-AcOH-H_2O, 12), 333(100), 292(90).

24-Hydroxyglycyrrhetic acid (II). MS *m/z* (rel. int.): 486(M^+, 17), 468(M^+-H_2O, 45), 439(67), 303(100), 262(74), 173(31), 135(62). ^{1}H-NMR (270 MHz, CD_3OD): δ0.82(3H, s, Me), 1.11(6H, s,

Me × 2), 1.13(3H, s, Me), 1.20(3H, s, Me), 1.41(3H, s, Me), *ca.* 3.2(1H, overlapped with the solvent peak, H-3), 3.40(1H, d, J = 11.0 Hz, Ha-25), 4.09(1H, d, J = 11.0 Hz, Hb-25), 5.63(1H, s, H-12).

Isolation of a hydroxylation product of 3-oxoGA

Liver microsomes of female rats (15 weeks old) were used for the hydroxylation of 3-oxoGA, because of no reductive activity of 3-oxoGA to GA in microsomes of female rats in spite of the potent reductive activity in those of male rats (unpublished data). The reaction mixture contained 480 mg of microsomal protein, 30 μmol of 3-oxoGA and 500 μmol of NADPH in 300 mL of 0.1 M potassium phosphate buffer (pH 7.2). A hydroxylation product III was isolated in the same way as described above. In this case one major product was obtained on TLC.

22α-Hydroxy-3-oxo-glycyrrhetic acid (III). MS *m/z* (rel. int.): 466(M^+-H_2O, 3), 420(22), 418(24), 403(19), 301(15), 253(100), 213(24), 159(16). ^{1}H-NMR (400 MHz, CD_3OD): δ0.94(3H, s, H_3-28), 1.06(3H, s, H_3-24), 1.09(3H, s, H_3-23), 1.11(3H, s, H_3-29), 1.17(3H, s, H_3-26), 1.24(3H, s, H_3-25), 1.44(3H, s, H_3-27), 2.14(1H, br d, J = 13.1 Hz, H-15), 2.31(1H, br d, J = 10.7 Hz, Hβ-18), 2.55(1H, dd, J = 9.9, 7.5 Hz, Hβ-2), 2.59(1H, s, Hα-9), 2.83(1H, dd, J = 13.6, 7.5 Hz, H-1), 3.50(1H, dd, J = 12.1, 3.8 Hz, Hβ-22), 5.73(1H, s, H-12).

RESULTS AND DISCUSSION

Presence of GA-metabolizing activity in rat liver

Liver homogenates of male rats oxidized GA to 3-oxoGA in the presence of $NADP^+$ [11], but did not produce any other metabolites. However, in the presence of NADPH two metabolites were obtained in addition to a small amount of 3-oxoGA, which might be produced by $NADP^+$ derived from NADPH. These metabolites, I and II, were produced more effectively by the postmitochondrial supernatant. Both metabolites had lower R_f values on TLC than GA and I showed slightly lower R_f value than II (Fig. 1).

Microsomal localization of I- and II-producing activities

The metabolizing activities from GA to I and II were localized in the same microsomal fraction as GA dehydrogenase activity [11], judged from their subcellular distributions as shown in Fig. 2. There were no loss of the activities during washing the microsomes with 1.15% KCl containing 10 mM ethylenediaminetetraacetic acid (EDTA) and 1.0 M NaCl, so that the GA-metabolizing activities are ascribed to microsomal enzymes, but not to the adsorbed enzymes to the membrane.

NADPH and NADH were required for production of both I and II, and the former showed higher activity than the latter, but the addition of $NADP^+$ and NAD^+ were inactive for both activities (Table 1). Under N_2 atmosphere, production of both I and II decreased remarkably even by the addition of NADPH. The addition of a supernatant fraction did not affect these microsomal activities. Optimal pHs of both activities were around neutral showing the broad peak. Moreover, both activities were strongly inhibited by CO (Table 2).

These results suggest that I and II are hydroxylated derivatives which are produced by microsomal oxygenation system including cytochrome P450s.

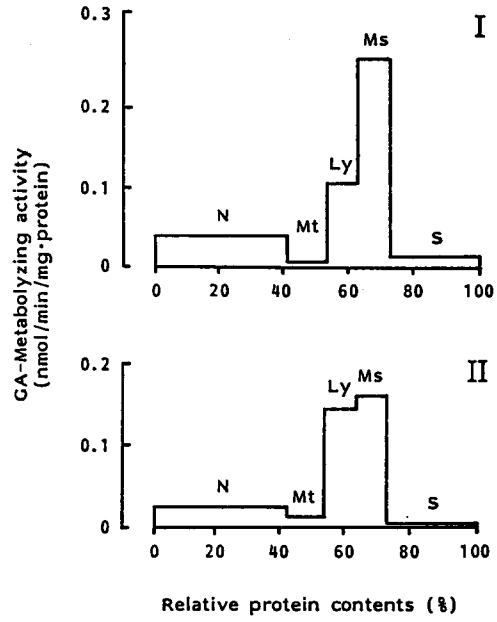

Fig. 2. Subcellular distribution of GA-metabolizing activities in male rat liver. The ordinate indicates the specific activity metabolizing GA to I (top panel) and II (bottom panel) per mg of protein. The subcellular fractions are shown on the abscissa by their relative protein contents in the order of their isolation. N, Mt, Ly, Ms and S represent the nuclear, mitochondrial, lysosomal, microsomal and soluble fractions, respectively.

Table 1. Cofactor requirement for GA-metabolism

Cofactors	Metabolite produced (nmol/min/mg) I	II
NADPH	0.49	0.24
NADPH under N_2 gas	0.12	0.052
NADH	0.078	0.080
$NADP^+$	<0.02	<0.02
NAD^+	<0.02	<0.02

Table 2. Inhibition of GA-metabolism by CO

O_2:CO	Inhibition (%)* I	II
20:80	67.5	43.5
10:90	83.5	52.6

* Activities in 20:80 of O_2:N_2 against 20:80 of O_2:CO and in 10:90 of O_2:N_2 against 10:90 of O_2:CO were measured as a respective control. Values are represented as percentage inhibition against the respective control.

Table 3. ^{13}C-NMR spectral data for metabolites and 3-oxoGA

	I*	II*	III*‡	3-oxoGA†‡
1	41.0 (t)	39.9 (t)	41.5 (t)	41.5 (t)
2	28.1 (t)	34.0 (t)	35.6 (t)	35.7 (t)
3	80.2 (d)	80.7 (d)	221.0 (s)	220.7 (s)
4	40.1 (s)	43.4 (s)	31.5 (s)	33.6 (s)
5	57.0 (d)	56.5 (d)	56.9 (d)	56.8 (d)
6	19.4 (t)	17.2 (t)	20.7 (t)	20.6 (t)
7	34.6 (t)	32.8 (t)	33.8 (t)	33.7 (t)
8	46.4 (s)	43.4 (s)	47.4 (s)	47.2 (s)
9	63.9 (d)	62.9 (d)	63.1 (d)	62.9 (d)
10	39.1 (s)	37.8 (s)	38.6 (s)	38.5 (s)
11	203.5 (s)	202.4 (s)	202.7 (s)	202.4 (s)
12	129.8 (d)	128.6 (d)	129.7 (d)	129.6 (d)
13	173.2 (s)	173.6 (s)	173.7 (s)	173.8 (s)
14	44.5 (s)	46.0 (s)	46.1 (s)	45.6 (s)
15	28.6 (t)	28.1 (t)	28.2 (t)	28.3 (t)
16	21.3 (t)	27.4 (t)	21.2 (t)	29.6 (t)
17	39.1 (s)	32.8 (s)	39.2 (s)	33.6 (s)
18	51.1 (d)	¶	49.8 (d)	50.5 (d)
19	42.0 (t)	43.7 (t)	44.2 (t)	43.1 (t)
20	46.0 (s)	44.4 (s)	47.0 (s)	45.4 (s)
21	41.1 (t)	¶	41.7 (t)	32.7 (t)
22	77.9 (d)	39.3 (t)	77.9 (d)	39.7 (t)
23	29.4 (q)	23.0 (q)§	27.8 (q)	27.9 (q)
24	20.1 (q)	64.9 (t)	22.5 (q)	22.5 (q)
25	17.1 (q)	17.2 (q)	17.2 (q)	17.1 (q)
26	17.7 (q)	19.0 (q)	19.8 (q)	19.8 (q)
27	24.7 (q)	23.6 (q)§	24.6 (q)	24.6 (q)
28	26.7 (q)	29.1 (q)‖	26.8 (q)	28.1 (q)
29	29.5 (q)	29.3 (q)‖	30.4 (q)	30.0 (q)
30	181.5 (s)	179.9 (s)	181.2 (s)	181.0 (s)

* CD_3OD.
† CD_3OD—$CDCl_3$.
‡ Measured at 100 MHz.
All assignments were performed on the basis of ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, long-range ^{13}C-^{1}H COSY experiments.
§,‖ Assignments may be exchanged.
¶ Overlapped with solvent peaks.

Identification of I and II as 22α- and 24-hydroxyl GAs, respectively

Metabolite I was obtained as amorphous powder. Its ^{1}H-NMR spectrum showed the presence of seven *tert*-methyl groups, two hydroxy-bearing methine protons (δ3.23, dd, J = 11.7 and 4.6 Hz; δ3.55, dd, J = 12.1 and 4.5 Hz), and one olefinic proton. On acetylation followed by methylation with diazomethane, I yielded a methyl ester of the monoacetate, whose mass spectrum showed a molecular ion at m/z 542. These findings indicated that I was a monohydroxylated derivative of GA. The ^{1}H- and ^{13}C-NMR spectral data (Table 3) of I were similar to the reported values for 22-hydroxylated derivatives of GA [9]. By direct comparisons of the NMR spectra with those of an authentic sample, I was identified as 22α-hydroxyGA, which has been isolated as one of the metabolites of GA formed by *Streptomyces* [9].

Metabolite II was also obtained as an amorphous powder, whose mass spectrum showed a molecular ion at m/z 486. The ^{1}H-NMR spectrum showed the presence of six *tert*-methyl groups, one hydroxy-bearing methine proton (δca. 3.2), one olefinic proton (δ5.63), one hydroxymethyl group (δ3.40, d, J = 11.0 Hz; δ4.09, d, J = 11.0 Hz), indicating that one of the seven methyl groups present in GA was oxidized to a hydroxymethyl group in II. By comparing the ^{1}H-NMR and ^{13}C-NMR spectra (Table 3) with those of 22α,23- and 22α,24-dihydroxyGAs [9], the hydroxymethyl group was assigned to C-24, thus indicating that II is 24-hydroxyGA, which has been reported as a component of *Glycyrrhiza glabra* L. [15]. These results suggest that 22α- and 24-hydroxyGAs are produced *in vivo*, when GA is administered to rats. Unknown metabolites of GA in bile from rats, reported by Parke *et al.* [16] and Iveson *et al.* [5], may be 22α- and 24-hydroxyGAs and their conjugates.

Substrate specificity

By using the microsomes having high 22α- and low 24- or high 24- and low 22α-hydroxylation activity, the substrate specificity of each activity was examined. 22α- or 24-Hydroxylation was observed with 3-oxoGA, 3-epiGA and 18α-GA (Fig. 3), though metabolites of GL and GA monoglucuronide were not detected on TLC. 3-OxoGA was the best substrate for 22α-hydroxylation, and GA, 3-epiGA and 18α-GA was in the order of decreasing activity (Table 4). On the other hand, GA was hydroxylated at C-24 faster than 3-oxoGA, and both 3-epiGA, and 18α-GA were very poor substrates (Table 4). Many metabolites such as 22α-hydroxy-3-oxoGA, 24-hydroxy-3-oxoGA, GA, 22α-hydroxyGA and 24-hydroxyGA were produced from 3-oxoGA in the presence of NADPH by microsomes having two hydroxylation activities, because GA dehydrogenase catalysing the reversible reaction of GA and 3-oxoGA exists in microsomes of rat liver [11]. 22α-Hydroxy-3-oxoGA was also produced from GA in the presence of $NADP^+$ and NADPH. Results obtained from experiments of substrate specificity suggest that two kinds of cytochrome P450, which hydroxylate GA at 22α- and 24-position, are present in hepatic microsomes of rats.

22α-Hydroxy-3-oxoGA (III) was isolated and then identified as follows. III was obtained as amorphous powder. Its ^{1}H-NMR spectrum showed the presence of seven *tert*-methyl groups, a hydroxy-bearing methine (δ3.50, dd, J = 12.1 and 3.8 Hz) and an olefinic proton (δ5.73). In addition, the ^{13}C-NMR spectrum (Table 3) showed the signals at δ179.9 and 202.4 ascribable to a carboxy and an oxo group, which were present in the original compound, 3-oxoGA, (δ181.0, C-30; 202.4, C-3). The above findings revealed that III was a monohydroxylated derivative of 3-oxoGA. The mass spectrum, however, showed a weak dehydrated molecular ion at m/z 466. The ^{1}H-^{1}H shift correlated spectroscopy (COSY), ^{13}C-^{1}H COSY, long-range ^{13}C-^{1}H COSY and nuclear Oberhauser effect (NOE) experiments led to the complete assignments of the ^{13}C-NMR signals as shown in Table 3. The long-range shift correlation between the methyl signal at δ_H 0.94 (H_3-28) and the ^{13}C-signal at δ_C77.9 (d) suggested that III was either 16- or 22-hydroxy derivative. In a comparison of the ^{13}C-NMR spectra between III and 3-oxoGA, appreciable changes in chemical shift were observed

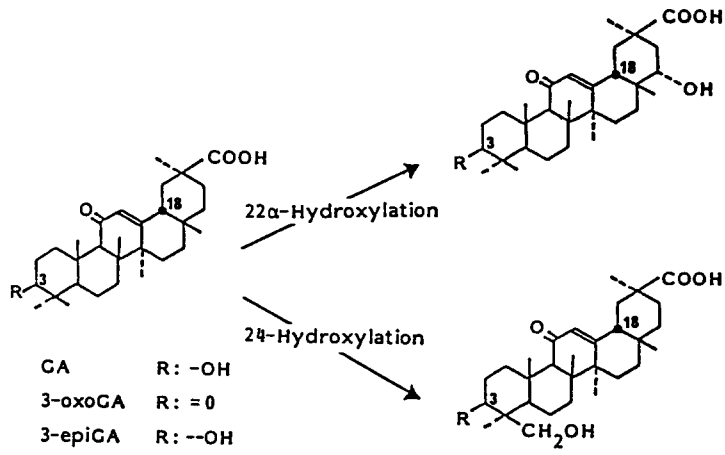

Fig. 3. Hydroxylation of GA and related compounds.

Table 4. Substrate specificity of 22α- and 24-hydroxylation

	22α-Hydroxylation (I)* (nmol/min/mg)	24-Hydroxylation (II)†
GA	0.30	0.10
3-oxoGA	0.50	0.050
3-epiGA	0.17	0.017
18α-GA	0.11	0.017

* Microsomes of female rats at age of 12 weeks were used.
† Microsomes of male rats at age of 12 weeks were used.

at C-16, C-17, C-20 and C-21 ($\Delta\delta$ −8.40, +5.60, +1.60 and +9.00, respectively) but no change at C-15, indicating that III is 22-hydroxy-3-oxoGA. Similar changes in the chemical shifts were also observed by 22-hydroxylation of GA. On the basis of the coupling constant for H-22 (dd, J = 12.1 and 3.8 Hz), the structure of III was finally established as 22α-hydroxy-3-oxoGA which is a new compound.

GA-hydroxylation activities may have an important role in the prevention of pseudoaldosteronism, one of the side effects of GL [17] and carbenoxolone [18], because hydroxylated products are easy to excrete generally. Since the mechanism of the pharmacological effects of GA is not verified, it is necessary to study the pharmacological effects of these hydroxylated metabolites.

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REFERENCES

1. Nakano N, Kato H, Suzuki H, Nakano N, Yano S and Kanaoka M, Enzyme immunoassay of glycyrrhetic acid and glycyrrhizin. II. Measurement of glycyrrhetic acid and glycyrrhizin in serum. *Jap Pharmacol Ther* **8**: 4171–4174, 1980 (in Japanese).
2. Hattori M, Sakamoto T, Kobashi K and Namba T, Metabolism of glycyrrhizin by human intestinal flora. *Planta Med* **48**, 38–42, 1983.
3. Hattori M, Sakamoto T, Yamagishi T, Sakamoto K, Konishi K, Kobashi K and Namba T, Metabolism of glycyrrhizin by human intestinal flora. II. Isolation and characterization of human intestinal bacteria capable of metabolizing glycyrrhizin and related compounds. *Chem Pharm Bull* **33**: 210–217, 1985.
4. Akao T, Akao T and Kobashi K, Glycyrrhizin β-D-glucuronidase of *Eubacterium* sp. from human intestinal flora. *Chem Pharm Bull* **35**: 705–710, 1987.
5. Iveson P, Lindup WE, Parke DV and Williams RT, The metabolism of carbenoxolone in the rat. *Xenobiotica* **1**: 79–95, 1971.
6. Downer HD, Galloway RW, Horwich L and Parke DV, The absorption and excretion of carbenoxolone in man. *J Pharm Pharmacol* **22**: 479–487, 1970.
7. Finney RSH and Somers GF, The anti-inflammatory activity of glycyrrhetinic acid and derivatives. *J Pharm Pharmacol* **10**: 613–620, 1958.
8. Tangri KK, Seth PK, Parmar SS and Bhargava KP, Biochemical study of anti-inflammatory and anti-arthritic properties of glycyrrhetic acid. *Biochem Pharmacol* **14**: 1277–1281, 1965.
9. Sakano K and Oshima M, Structures of conversion products formed from 18β-glycyrrhetinic acid by *Streptomyces* sp. G-20. *Agric Biol Chem* **50**: 763–766, 1986.
10. Sakano K and Oshima M, Microbial conversion of 18β-glycyrrhetinic acid and 22α-hydroxy-18β-glycyrrhetinic acid by *Chainia antibiotica*. *Agric Biol Chem* **50**: 1239–1245, 1986.

11. Akao T, Akao T and Kobashi K, Metabolism of glycyrrhetic acid by rat liver microsomes; glycyrrhetinate dehydrogenase. *Biochim Biophys Acta* **1042**: 241–246, 1990.
12. Imai Y, Ito A and Sato R, Evidence for biochemically different types of vesicles. *J Biochem* **60**: 417–428, 1966.
13. Kuriyama Y, Omura T, Siekevitz P and Palade GE, Effects of phenobarbital on the synthesis and degradation of the protein components of rat liver microsomal membranes. *J Biol Chem* **244**: 2017–2026, 1969.
14. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
15. Ganonica L, Danieli B, Manitto P, Russo G and Bonati A, Triterpenic della *Glycyrrhiza glabra*.—Nota IV. Acidi 24-idrossiglicirretico e 24-idrossi-11-desossoglicirretico. *Gazz Chim Ital* **97**: 1359–1369, 1967.
16. Parke DV, Pollock S and Williams LA, The fate of tritium-labelled β-glycyrrhetic acid in the rat. *J Pharm Pharmacol* **15**: 500–506, 1963.
17. Molhuysen JA, Gerbrandy J, de Vries LA, de Jong JC, Lenstra JB, Turner KP and Borst JGG, A liquorice extract with deoxycortone-like action. *Lancet* **2**: 135–137, 1950.
18. Horwich L and Galloway R, Treatment of gastric ulceration with carbenoxolone sodium: clinical and radiological evaluation. *Br Med J* **2**: 1274–1277, 1965.