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A BACTERIAL CLEAVAGE OF THE *C*-GLUCOSYL BOND OF MANGIFERIN AND BERGENIN

MASAO HATTORI,*† YUE-ZHONG SHU,† TSUYOSHI TOMIMORI,‡ KYOICHI KOBASHI§ and TSUNEO NAMBA†

†Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930, Japan; ‡School of Pharmacy, Hokuriku University, 3 Ho, Kanagawa-machi, Kanazawa, 920-11, Japan; §Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930, Japan

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Key Word Index—Bergenin, mangiferin, intestinal bacteria, *C*-glucosyl bond cleavage; biotransformation.

Abstract—Two *C*-glucosyls, mangiferin and bergenin, were transformed to the respective aglycones, 1,3,6,7-tetrahydroxyxanthone and 4-*O*-methylgallic acid, by a mixture of human intestinal bacteria.

INTRODUCTION

C-Glycosyls are a special type of glycosides since C-1 of the sugar ring is directly attached to the aglycone nucleus by a C–C bond and are known to occur as flavonoid *C*-glycosides, xanthone *C*-glycosides, chromone *C*-glycosides, anthrone *C*-glycosides and *C*-glycosylated gallic acids [1]. Their characteristic properties are resistance toward acid and enzymic hydrolysis in contrast with *O*-glycosides.

In our previous papers [2, 3], we have reported that a flavonoid glucoside, homoorientin, and an anthrone *C*-glucoside, barbaloin, are metabolized to *dl*-eryodictylol and aloe-emodin anthrone, respectively, by human intestinal flora.

In the present paper, we report additional evidence on the *C*-glucosyl bond cleavage in a xanthone *C*-glucoside, mangiferin (**1**), and a glucosylated gallic acid, bergenin (**2**) by human intestinal bacteria

RESULTS

Metabolism of mangiferin (**1**) *by human intestinal flora*

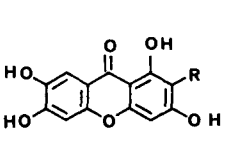
By incubation with a bacterial mixture from human faeces, mangiferin (**1**) was converted to a metabolite, a yellow powder, mp 300°. The UV spectrum showed λ_{max} at 236, 254, 312 and 360 nm, quite similar to that of the original compound. The high-resolution mass spectrum exhibited the molecular formula $C_{13}H_{18}O_6$, indicating that the *C*-glucosyl moiety in mangiferin (**1**) was eliminated by intestinal bacteria The metabolite was identified as 1,3,6,7-tetrahydroxyxanthone (northyriol, **3**) by comparing the IR, ^{1}H NMR, ^{13}C NMR and mass spectra with those of an authentic sample [4] The time course experiment indicated that no metabolite was detected in the initial 10 hours incubation, but the aglycone appeared at 12 hr (*ca* 10% in molar basis) and reached to a maximal amount at 36 hr (*ca* 94%). The further incubation up to 48 hr did not appreciably change the amount of the aglycone.

Metabolism of bergenin (**2**) *by human intestinal flora*

Similarly, a metabolite was formed by the incubation of bergenin (**2**) with a bacterial mixture from human faeces. The metabolite, colourless prisms, mp 261–262°, showed a chemical composition of $C_8H_8O_5$ by high-resolution mass spectrometry. The ^{1}H NMR and ^{13}C NMR spectra showed the presence of a methoxy group (^{1}H, δ 3.88; ^{13}C, δ 61.5) two magnetically equivalent aromatic protons (^{1}H, δ 7.08) and carboxyl carbon (^{13}C, δ 170.6) but the absence of a glucose moiety. These findings led us to conclude the metabolite as 4-*O*-methylgallic acid (**4**). When this cleavage reaction was monitored by TLC-densitometry, the metabolite began to appear 18 hr after incubation (22% in molar basis), reached to a maximal concentration at 30–36 hr (92%), and then gradually decreased (64% at 48 hr)

DISCUSSION

With regard to the metabolism of mangiferin (**1**) [5, 6], it was reported that oral administration of mangiferin (**1**) to rabbits resulted in the urinary excretion of euxanthone



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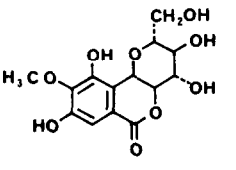
3 R = H



2

*Author to whom correspondence should be addressed.

and its 7-glucuronide (euxanthic acid) [5, 6]. This unusual metabolite formation involves the removal of the *C*-glucosyl residue and the two phenolic hydroxyl groups They did not ascertain the site of this interesting reaction, but Scheline [7] pointed out the possibility that the gut flora might be responsible for the elimination of the *C*-glucosyl residue Our experiment reveals that intestinal flora promote the cleavage reaction of the *C*-glucoside in mangiferin

On the other hand, Minamikawa *et al.* [8] reported that *Erwinia herbicola*, a strain of soil bacteria isolated from the rhizosphere of *Bergenia crassifolia*, degraded bergenin (**2**) to give 4-*O*-methylgallic acid (**4**) when the bacterium was cultured in the presence of bergenin (**2**) as a sole carbon source Therefore, our result provide an additional example of bacterial degradation of bergenin (**2**) via a *C*-glucosyl bond cleavage

EXPERIMENTAL

General Mps uncorr ^{1}H and ^{13}C NMR spectra were measured with TMS as int. std MS were measured at 70 eV (EIMS probe) Wakogel C-200 was used for CC and Merck Kieselgel 60 F_{254} was used for TLC with the solvent system, $CHCl_3$–MeOH–AcOH–H_2O (72 20 3 2) Spots on the plates were detected under a UV lamp or by spraying with $FeCl_3$ reagent

Chemicals Mangiferin (**1**) and bergenin (**2**) were isolated from *Swertia chirata* Buch.-Ham [4] and *Astilbe thunbergii* Miquel [9], respectively A dilution medium for anaerobic bacteria was prepared according to the procedure of Mitsuoka [10] It contained the following 37 5 ml of soln A (0 78% K_2HPO_4), 37 5 ml of soln B (0 47% KH_2PO_4–1 18% NaCl–1 2% $(NH_4)_2SO_4$–0 12% $CaCl_2$–0 25% $MgSO_4$ H_2O), 1 ml of 0.1% resazurine, 0.5 g of L-cysteine HCl H_2O, 2 ml of 25% L-ascorbic acid, 50 ml of 8% Na_2CO_3 and H_2O to give a final volume of 1000 ml

Preparation of a human intestinal bacterial mixture Fresh faeces obtained from a healthy man was thoroughly suspended in 30 vol of the anaerobic dilution medium by bubbling with oxygen-free CO_2 and filtered through gauze to eliminate the residue The bacterial suspension thus obtained was used in the following expt

Incubation of mangiferin (**1**) *with an intestinal bacterial mixture* Mangiferin (**1**) (150 mg) was added to an intestinal bacterial mixture (200 ml) and anaerobically incubated for 36 hr at 37° in an anaerobic jar, replacing air with an oxygen-free CO_2 in the presence of activated steel wool (steel wool method) (**10**) The mixture was acidified with 3 M HCl and extracted with EtOAc (200 ml × 2) The organic layer was washed with H_2O and evapd *in vacuo* to give an oily residue The residue was chromatographed on a silica gel column (42 × 2 6 cm), which was eluted with successively hexane, $CHCl_3$, $CHCl_3$–MeOH (100 1) and $CHCl_3$–MeOH (50 1) A fraction eluted with the last solvent was concd to dryness *in vacuo* The residue was subjected to crystallization to yield yellow crystals (24 mg) mp 300°, high-resolution MS Found, 260 0349, Calcd for M^+, $C_{13}H_8O_6$, 260 0321, UV λ_{max}^{EtOH} (log ε) nm 238 (3 98), 256 (3 99), 311 (3.93), 362 (3 86) IR ν_{max}^{KBr} cm^{-1} 3270 (OH), 1620 (conjugated C=O), 1480 (Ar ring), ^{1}H NMR (270 MHz, DMSO-d_6) δ 6 13 (1H, *d*, *J* = 1 8 Hz, 2-H), 6 30 (1H, *d*, *J* = 1 8 Hz, 4-H), 6 84 (1H, *s*, 5-H), 7 38 (1H, *s*, 8-H), 10.50 (1H, *br s*, OH), 13 16 (1H, *s*, 1-OH), ^{13}C NMR (DMSO-d_6) δ 93 6 (*d*, C-4), 97 7 (*d*, C-2), 101 6 (*s*, C-8b), 102 7 (*d*, C-5), 108.1 (*d*, C-8), 111 8 (*s*, C-8a), 143 8 (*s*, C-7), 151 0 (*s*, C-4b), 154 1 (*s*, C-6), 157 3 (*s*, C-4a), 162 7 (*s*, C-1), 164 7 (*s*, C-3), 178 9 (*s*, C=O), MS *m/z* 260 (M^+, base peak), 232, 203, 152, 116, 69 The compound was identified as norathyriol (1,3,6,7-tetrahydroxyxanthone) (**3**)

Incubation of bergenin (**2**) *with an intestinal bacterial mixture* Bergenin (**2**) (100 mg) was anaerobically incubated with an intestinal bacterial mixture (200 ml) under the conditions similar to those of mangiferin (**1**) The EtOAc extract was chromatographed on a silica gel column (24 × 2 4 cm) with $CHCl_3$–MeOH (100 1) and a metabolite (73 mg) was isolated as colourless prisms (from $CHCl_3$–MeOH) mp 261–262°, high-resolution MS Found, 184 0388, Calcd for $C_8H_8O_5$, 184 0372 (M^+), UV λ_{max}^{EtOH} (log ε) nm 224 (3 83), 261 (3.81), sh 296 (3 40), IR ν_{max}^{KBr} cm^{-1} 3170 (OH), 1710 (C=O), 1595, 1510 (Ar ring), 1230 (COO), ^{1}H NMR (90 MHz, CD_3OD) δ 3 88 (3H, *s*, MeO), 7 08 (2H, *br s*, 2, 6-H), ^{13}C NMR (CD_3OD) δ 61 5 (*q*, OMe), 111 2 (*d*, C-2 and C-6), 127 7 (*s*, C-1), 141 7 (*q*, C-4), 152 1 (*s*, C-3 and C-5), 170 6 (*s*, COOH), MS *m/z* 184 (M^+), base peak, 169 [M – 15], 141, 113, 67 The metabolite was identified as 4-*O*-methylgallic acid (**4**)

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