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BIOTRANSFORMATION OF AN ACYCLIC NEOLIGNAN IN RATS

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Abstract—The biotransformation of an acyclic neolignan, (+)-*erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′neolignan, in rats has been investigated. After administration of (+)-*erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan to rat by intraperitoneal injection, urine and faeces were collected. A small amount of (+)-*erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan and its metabolic product were obtained from an ethyl acetate extract of the urine, and the largest amount of the same metabolic product was obtained from a dichloromethane extract of the faeces. The sole metabolic product was identified as (+)-(4-hydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan by spectroscopic methods. Furthermore, biotransformation of (+)-*erythro*-(4,7-dihydro-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan by intestinal bacteria in rat faeces was also investigated *in vitro*. Consequently, (+)-*erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan was reduced to the same metabolic product and no other metabolic products were produced. These results suggested that intestinal bacteria were concerned in the specific dehydroxylation of (+)-*erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan in rats.

INTRODUCTION

Lignans and neolignans are widely distributed in terrestrial plants and exhibit various biological activities against insect and microorganisms, e.g. Licarin A and (−)-machilusin inhibit the growth of *Spodoptera litura* larvae [1], (+)-epimagnolin A inhibits the growth of larvae of *Drosophila melanogaster* [2] and magnolol shows potent antibacterial activity against a primary carcinogenic bacterium, *Streptococcus mutans* [3]. They also have pharmacological activities, e.g. magnosalicin shows an anti-allergy effect [4] and (+)-pinoresinol inhibits the activity of cyclic AMP phosphodiesterase [5]. Therefore, the generation of bioactive lignan derivatives and the elucidation of their metabolic pathways in animals and microbes are important.

As part of our continuous programme to investigate biotransformation of lignans and neolignans, the biotransformation of (+)-magnolin and (+)-yangabin in rats [6] and the microbial transformation of (+)-eudesmin, (+)-magnolin and (+)-epimagnolin by *Aspergillus niger* [7, 8] have been investigated. Consequently, we obtained several new lignans and revealed that the first metabolic reaction in rats and *A. niger* was specific de-*O*-methylation at the *p*-position. In these metabolic pathways, however, oxidation only occurred at their aryl groups and no oxidation occurred at their cyclic side chain. This fact led us to examine the biotransformation of acyclic lignans and neolignans, to elucidate their metabolic pathways and to generate their derivatives.

(+)-*Erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan (**1**) is an acyclic 8-*O*-4′-neolignan isolated from the aril [9, 10] or the seeds of *Myristica fragrans* [11, 12]; the absolute configuration of **1** was assigned by comparison of spectral data with its derivatives [13]. A derivative of **1** has antifeeding activity against silkworm larvae, (*Bombyx mori*) [11], and therefore, production of its derivatives and investigations on their biological activities are important. The present paper deals with the biotransformation of **1** to (+)-(4-hydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan (**2**) in rats and the biotransformation of **1** to **2** by intestinal bacteria in rat faeces *in vitro*.

RESULTS AND DISCUSSION

(+)-*Erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan (**1**) was administered to five male rats by intraperitoneal injection and faeces and urine were collected for 2 days. The gas chromatogram of a CH_2Cl_2 extract of **1**-administered rat faeces contained a novel peak of the faecal metabolic product of **1** compared with that of the control; however, no peaks corresponding to **1** on other metabolic products were detected. This faecal metabolic product **2** was also detected on TLC (R_f 0.64)

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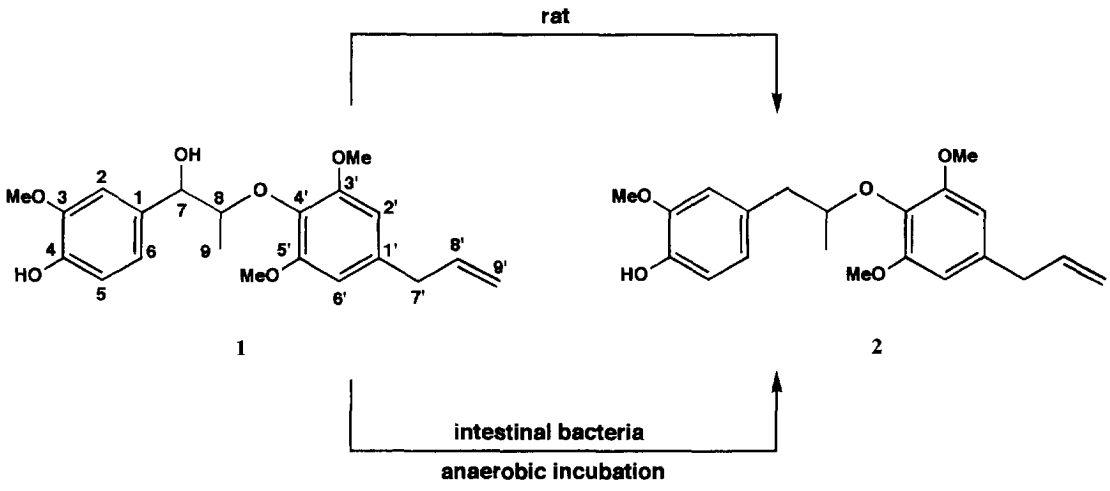

developed with $CHCl_3$–Me_2CO (9:1). The CH_2Cl_2 extract was washed with 5% $NaHCO_3$ solution, subjected to silica gel column chromatography and purified by preparative. TLC to give faecal product **2**. Product **2** had a molecular formula of $C_{21}H_{26}O_5$ as determined by high resolution mass spectral and NMR data. The IR spectrum exhibited a hydroxyl band at 3491 cm^{-1} and (+)-specific optical rotation. The mass spectrum showed specific ions at *m/z* 358 $[M]^+$, 194, 165 and 137. The ^1H NMR spectral data corresponded with those of **1** except for the disappearance of hydroxyl and methine proton signals at the 7-position and the appearance of new methylene protons at δ2.72 (*dd*, *J* = 8.5, 13.5 Hz) and 3.11 (*dd*, *J* = 5, 13.5 Hz). The ^{13}C NMR spectrum also showed a new signal for the C-7 benzyl methylene carbon at δ42.9. Therefore, the specific ions at *m/z* 194 $[R + H]^+$ (R = 1-allyl-3,5-dimethoxyphenoxyl group), *m/z* 165 $[ArCH_2CHMe]^+$ and 137 $[ArCH_2]^+$ (Ar = 4-hydroxy-3-methoxyphenyl group) in the mass spectrum could be assigned. These spectral data allowed us to identify **2** as (+)-(4-hydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan.

The **1**-administered rat urine was extracted with EtOAc, then extracted with CH_2Cl_2 after incubation with β-glucuronidase for 48 hr at 37°. The gas chromatogram of the EtOAc extract contained peaks corresponding to **1** and **2**. The structures of urinary metabolic products **1** and **2** were confirmed by GC-mass spectrometry. The gas chromatogram of the CH_2Cl_2 extract of **1**-administered rat urine contained no peaks for the metabolic product of **1**.

These results suggested that the metabolism of **1** in rats was regiospecific dehydroxylation at 7-position. Biotransformation of the acyclic neolignan, magnolol, in rats has been previously investigated [14] and shown to involve reduction and isomerization of allyl groups. With regard to the biotransformation of a neolignan which has an allyl group, the metabolism of kadsurenone in Rhesus monkey and rat liver microsomes has been reported [15] as oxidation of the allyl group. However, no metabolism of the allyl group of **1** occurred in rats, instead, a specific reduction proceeded.

With regard to the metabolic route in rats, the greatest amount of metabolic product **2** was excreted in faeces (730 mg) with only a small amount (< 10 mg) in urine together with **1** (< 10 mg). This result suggested that intestinal bacteria were responsible for the specific dehydroxylation of **1**. Therefore, in order to clarify whether this reduction was mediated by intestinal bacteria, biotransformation of **1** by intestinal bacteria in rats was examined *in vitro*. Fresh rat faeces were suspended in physiological saline and incubated with **1** under anaerobic conditions in GAM medium at 37° for 2 days. Compound **1** was metabolized and reduced to **2** without producing by-products. This clearly revealed that intestinal bacteria were implicated in the metabolism of **1** to **2** in rats.

The dehydroxylation of benzyl alcohols by intestinal microflora has been investigated by Scheline [16]. Benzyl alcohol derivatives containing a *p*-hydroxy group, or those capable of obtaining such a group through *O*-demethylation, were reduced to the corresponding toluene derivatives. This suggested that specific reduction occurred by dehydration to give a quinone methide, followed by subsequent hydrogenation. There are three possible routes for the dehydroxylation of **1**. The first is direct reduction of an oxygen atom to give the corresponding methylene. The second is dehydration to give a C-7,8 double bond followed by specific hydrogenation. The last route is dehydration to give a quinone methide followed by reduction. The latter route is probably correct in the dehydroxylation of **1** in rat. However, we could not elucidate the precise metabolic route because no intermediates were obtained in our experiments.

This investigation has revealed that (i) the metabolism of **1** in rats was specific dehydroxylation at the 7-position with no metabolism of the allyl group, (ii) the greatest amount of metabolic product **2** was excreted in faeces with a small amount excreted in urine with **1**, (iii) no glucuronide of the metabolic product of **1** was obtained from urine, (iv) intestinal bacteria were concerned with the specific dehydroxylation of **1** in rats and (v) the metabolic product **2** was a new neolignan generated by biotransformation.

EXPERIMENTAL

Prepn of neolignan. (+)-*Erythro*-(4,7-dihydroxy-3-methoxy-1′-allyl-3′,5′-dimethoxy)-8-*O*-4′-neolignan (**1**) was isolated from seeds of *Myrisitica fragrans* by previously reported methods [11].

Administration of (**1**). Five male rats (body wt 350 to 400 g) were fasted for 1 day before intraperitoneal injection of **1** (2.3 g) dissolved in DMSO. Rats were housed in metabolism cages and fed rat food and H_2O. Faeces were collected for 2 days at 24 hr intervals and stored at $-10°$ until examined. Urine was collected for 2 days (under toluene) and stored at $-10°$ until examined.

Extraction of metabolites from faeces. Faeces (132 g) were extracted (× 3) with MeOH (300 ml) and the solvent evapd under red. pres. The MeOH extract was suspended in H_2O, extracted (× 3) with CH_2Cl_2 and the solvent evapd under red. pres; 5.87 g of CH_2Cl_2 extract was obtained from the **1**-administered rat faeces.

Extraction of metabolites from urine. Urine (800 ml) was adjusted to pH 3 with 1 M HCl and then extracted with Et_2O, repeatedly. Acetate buffer was added and the pH adjusted to 4.5 and 50 mg of β-glucuronidase was added. After incubation for 48 hr at 37°, the urine was extracted with CH_2Cl_2 for 48 hr. The CH_2Cl_2 layer was evapd under red. pres.

Isolation of (**2**) *from faeces.* The CH_2Cl_2 extract (5.87 g) of the **1**-administered faeces was dissolved in CH_2Cl_2, washed with 5% $NaHCO_3$ soln and sepd into acidic (1.3 g) and non-acidic parts (4.5 g). The non-acidic part was subjected to silica gel CC with hexane containing increasing concns of $CHCl_3$ (10–50%) and purified by prep. TLC. This gave **2** as the sole metabolic product of **1** (730 mg). No metabolic product of **1** was obtained from the acidic part.

Table 1. ^{1}H NMR spectral data of neolignans **1** and **2**

H	1	2
2	6.97 *d* (2)	6.77 *d* (2)
5	6.83 *d* (8)	6.81 *d* (8)
6	6.67 *dd* (2, 8)	6.69 *dd* (2, 8)
7	4.79 *d* (2.5)	2.72 *dd* (8.5, 13.5)
	—	3.11 *dd* (5, 13.5)
8	4.34 *ddd* (3, 6.5, 13)	4.33 *m*
9	1.12 *d* (6.5)	1.20 *d* (6)
2′	6.46 *s*	6.40 *s*
6′	6.46 *s*	6.40 *s*
7′	3.36 *d* (6.5)	3.34 *d* (7)
8′	5.98 *m*	5.97 *m*
9′	5.11 *ddd* (1.5, 3, 10)	5.08 *ddd* (1, 3, 9)
	5.13 *ddd* (2, 3.5, 17)	5.11 *ddd* (2, 3.5, 17)
OH		
4-	5.64 *s*	5.48 *s*
7-	4.12 *br s*	—
OMe		
3-	3.88 *s*	3.86 *s*
3′-	3.86 *s*	3.79 *s*
5′-	3.86 *s*	3.79 *s*

Recorded in $CDCl_3$ at 500 Hz. Chemical shifts in ppm downfield from TMS. Coupling constants in Hz.

Table 2. ^{13}C NMR spectral data of neolignans **1** and **2**

C	1 (500 MHz)	2 (270 MHz)
1	133.0	134.3
2	108.6	112.2
3	146.4	146.1
4	144.5	143.8
5	113.9	113.9
6	118.8	122.1
7	82.3	42.9
8	72.8	80.0
9	12.7	19.5
1′	136.1	135.4
2′	105.5	105.5
3′	153.4	153.6
4′	132.0	131.0
5′	153.4	153.6
6′	105.5	105.5
7′	40.5	40.5
8′	137.0	137.3
9′	116.1	115.9
OMe		
3-	55.9	55.9
3′-	56.1	56.0
5′-	56.1	56.0

Chemical shifts in ppm downfield from TMS.

Incubation of (**1**) *with faeces.* Fr. rat faeces (200 mg) were suspended in 5 ml of physiological saline. An aliquot of the suspension (0.5 ml) was added to GAM medium (5 ml) containing 0.2 mg of **1** and incubated under anaerobic conditions at 37° for 2 days. The medium was extracted with Et_2O (× 3) and the solvent evapd under red. pres. The Et_2O extract was analysed by GC and GC-MS.

(+)-*Erythro*-(4,7-*dihydroxy*-3-*methoxy*-1′-*allyl*-3′,5′-*dimethoxy*)-8-O-4′-*neolignan* (**1**). $[\alpha]_D^{19}$ + 1.02 ($CHCl_3$; *c* 12.4). EIMS (GC, 70 eV) *m/z* (rel. int.): 374 $[M]^+$ (2), 356 (2), 221 (10), 194 (100), 179 (4), 164 (6), 151 (4), 131 (3), 91 (4), 83 (18). IR ν_{max}^{KBr} cm^{-1}: 3497 (OH), 3004, 2980, 2939, 2841, 1639, 1591, 1517, 1504, 1464, 1426, 1272, 1241, 1229, 1126, 1036. NMR data: Tables 1 and 2.

(+)-(4-*Hydroxy*-3-*methoxy*-1′-*allyl*-3′, 5′-*dimethoxy*)-8-O-4′-*neolignan* (**2**). $[\alpha]_D^{19}$ + 2.35 ($CHCl_3$; *c* 1.0). HRMS *m/z*: 358.1797 ($[M]^+$, calcd for $C_{21}H_{26}O_5$: 358.1781). EIMS (GC, 70 eV) *m/z* (rel. int.): 358 $[M]^+$ (2), 256 (18), 194 (58), 165 (100), 137 (11), 103 (10). IR ν_{max}^{KBr} cm^{-1}: 3491 (OH), 2935, 2839, 1589, 1516, 1504, 1463, 1423, 1270, 1240, 1037. NMR data: Tables 1 and 2.

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