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(+)-VINIFEROL D, A NEW STILBENETRIMER FROM THE STEM OF *VITIS VINIFERA* 'KYOHOU'

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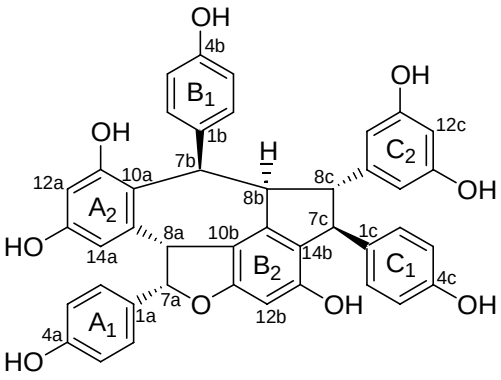
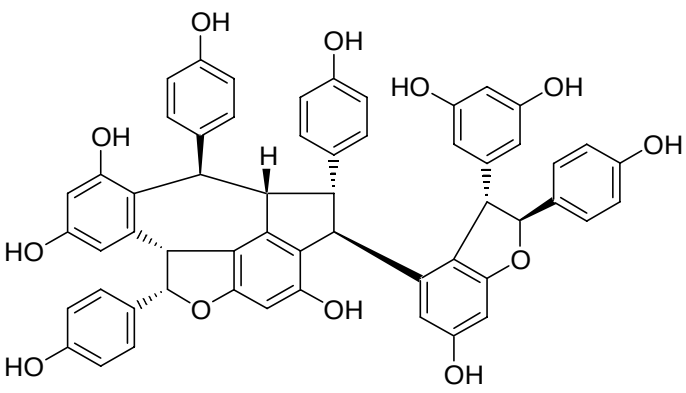
Abstract – A new stilbenetrimer named (+)-viniferol D having a bicyclo[5.3.0]decane ring system was isolated from the stem of *Vitis vinifera* 'Kyohou' and the structure was elucidated on the basis of the spectral evidence. The biogenesis was also discussed.

INTRODUCTION

In the previous papers, we reported the isolation and structures of stilbenetetramers having a bicyclo[6.3.0]undecane ring system ((+)-viniferol A), and having a bicyclo[5.3.0]decane ring system ((+)-viniferol B and (+)-viniferol C), from the stem of *Vitis vinifera* 'Kyohou' cultivated in Wakayama Prefecture, Japan.[2,3] Our further study of the constituents of the above plant led to the isolation of a new stilbenetrimer named (+)-viniferol D. In this paper, we describe the isolation and structural elucidation of a new stilbenetrimer having a bicyclo[5.3.0]decane ring system from the stem of *V. vinifera* 'Kyohou'.



(+)-Viniferol D (**1**)



(+)-Viniferol C (**2**)

RESULTS AND DISCUSSION

Isolation

The ethyl acetate soluble fraction described in the previous papers[2,3] of the stem of *V. vinifera* 'Kyohou' was fractionated by medium-pressure column chromatography (MPCC) using silica gel to give thirteen fractions. The fraction (F10) including (+)-viniferol C (**2**)[3] was successively separated by MPCC using reversed phase silica gel (C-8), by column chromatography (CC) using Sephadex LH-20, and by preparative TLC to give (+)-viniferol D (**1**) together with (+)-viniferol C (**2**).

Structure of (+)-viniferol D

(+)-Viniferol D (**1**), $[\alpha]_D$ +101.4° (*c* 0.27, MeOH) was found to have the molecular formula $C_{42}H_{32}O_9$ determined by high resolution FABMS. The ^{1}H NMR spectrum in methanol-d_4 of **1** exhibited signals for three sets of AA´XX´ type (1,4-disubstituted) aromatic hydrogens at δ 7.10 and 6.69 (each 2H, d, *J* = 8.8 Hz); 6.94 and 6.52 (each 2H, d, *J* = 8.8 Hz); 6.69 and 6.54 (each 2H, d, *J* = 8.8 Hz), one set of *meta*-coupled aromatic hydrogens at δ 6.25 and 6.41 (each 1H, d, *J* = 2.2 Hz), and one set of AX_2 type *meta*-coupled aromatic hydrogens at δ 6.30 (2H, d, *J* = 2.2 Hz) and 6.11 (1H, t, *J* = 2.2 Hz), and an uncoupled aromatic hydrogen at δ 5.99 (1H, s), as shown in Table 1. These were in good accordance with (+)-viniferol D being a trimer of resveratrol (3,5,4´-trihydroxystilbene). The signals for one set of aliphatic hydrogens at δ 6.02 and 4.27 (each 1H, d, *J* = 2.9 Hz) suggested the presence of one dihydrobenzofuran moiety bearing 4-oxyphenyl and 3,5-dioxyphenyl groups characteristic of oligostilbenes derived from the resveratrol molecule. The four aliphatic hydrogens at δ 5.12 (1H, br s, H-7b),[4] 4.18 (1H, d, *J* = 11.7 Hz, H-8b),[4] 2.87 (1H, dd, *J* = 11.7, 9.9 Hz, H-8c) and 4.30 (1H, d, *J* = 9.9 Hz, H-7c) suggested (+)-viniferol D (**1**) to be like (+)-ampelopsin C (**3**)[5] having a bicyclo[5.3.0]decane ring system. This was further confirmed by the following spectral data. The correlations of the HMBC spectrum were observed between H-2a (6a) and C-7a, between H-7a and C-9a, between H-7b and C-9a, C-11a, C-1b, C-9b, between H-12b and C-10b, C-14b, between H-8c and C-9c, C-10c (14c), and between H-7c and C-1c, respectively. The discussion above indicated (+)-viniferol D (**1**) to be a stereoisomer of (+)-ampelopsin C (**3**).[5] The ^{13}C NMR data of **1** are certainly very close to those of **3**, as shown in Table 1. The stereochemistry of **1** was estimated on the basis of the ^{1}H NMR spectral data, as follows. The chemical shift value of H-8a (δ 4.27) indicated the stereochemistry between H-8a and H-7b to be *anti*, as shown in the cases of (+)-ampelopsin C (**3**)[5] (δ 4.31) and (+)-viniferol B (**4**)[3] (δ 4.25).[6] It caused such an upfield shift that H-8a is held in the shielding region of the aromatic group (B_1) at C-7b.[7,8] Furthermore, the stereochemistry of **1** was respectively confirmed by the NOESY and DIFNOE experiments. The cross-peaks observed between H-7b and H-10c (14c), between H-8b and H-10c (14c) and between H-7c and H-10c (14c) suggested, respectively, the stereochemistry between H-7b and H-8b,

Table 1. The NMR Data of **1** and **3** in CD_3OD

	1		**3**	
No.	C	H	C	H
1a	134.6 (s)		130.8 (s)	
2a, 6a	128.0 (d)	7.10 (2H, d, *J* = 8.8 Hz)	130.4 (d)	7.16 (2H, d, *J* = 8.4 Hz)
3a, 5a	116.2 (d)	6.69 (2H, d, *J* = 8.8 Hz)	116.3 (d)	6.74 (2H, d, *J* = 8.4 Hz)
4a	158.3 (s)		158.96 (s)	
7a	86.5 (d)	6.02 (1H, d, *J* = 2.9 Hz)	91.2 (d)	5.75 (1H, d, *J* = 11.7 Hz)
8a	51.0 (d)	4.27 (1H, d, *J* = 2.9 Hz)	48.7 (d)	4.31 (1H, d, *J* = 11.7 Hz)
9a	147.4 (s)		142.0 (s)	
10a	118.6 (s)		125.9 (s)	
11a	158.7 (s)		156.3 (s)	
12a	101.4 (d)	6.25 (1H, d, *J* = 2.2 Hz)	101.8 (d)	6.22 (1H, d, *J* = 2.6 Hz)
13a	157.1 (s)		156.9 (s)	
14a	103.7 (d)	6.41 (1H, d, *J* = 2.2 Hz)	105.9 (d)	6.00 (1H, d, *J* = 1.8 Hz)
1b	138.0 (s)		134.1 (s)	
2b, 6b	129.8 (d)	6.94 (2H, d, *J* = 8.8 Hz)	130.9 (d)	7.13 (2H, d, *J* = 8.8 Hz)
3b, 5b	115.5 (d)	6.52 (2H, d, *J* = 8.8 Hz)	115.6 (d)	6.64 (2H, d, *J* = 8.8 Hz)
4b	155.8 (s)		155.8 (s)	
7b	37.1 (d)	5.12 (1H, br s)	37.9 (d)	5.18 (1H, d, *J* = 3.3 Hz)
8b	51.9 (d)	4.18 (1H, d, *J* = 11.7 Hz)	52.8 (d)	3.67 (1H, br d, *J* = 11.7 Hz)
9b	143.8 (s)		144.7 (s)	
10b	119.5 (s)		116.6 (s)	
11b	159.6 (s)		159.5 (s)	
12b	96.2 (d)	5.99 (1H, s)	96.8 (d)	6.09 (1H, s)
13b	154.9 (s)		154.9 (s)	
14b	123.0 (s)		121.5 (s)	
1c	135.1 (s)		133.4 (s)	
2c, 6c	130.1 (d)	6.69 (2H, d, *J* = 8.8 Hz)	130.3 (d)	6.96 (2H, d, *J* = 8.4 Hz)
3c, 5c	116.0 (d)	6.54 (2H, d, *J* = 8.8 Hz)	116.0 (d)	6.68 (2H, d, *J* = 8.4 Hz)
4c	156.5 (s)		156.8 (s)	
7c	57.2 (d)	4.30 (1H, d, *J* = 9.9 Hz)	58.4 (d)	4.17 (1H, d, *J* = 9.2 Hz)
8c	68.1 (d)	2.87 (1H, dd, *J* = 11.7, 9.9 Hz)	62.9 (d)	3.78 (1H, dd, *J* = 11.7, 9.2 Hz)
9c	144.6 (s)		147.4 (s)	
10c,14c	108.7 (d)	6.30 (2H, d, *J* = 2.2 Hz)	107.9 (d)	6.14 (2H, d, *J* = 2.2 Hz)
11c,13c	158.9 (s)		159.04 (s)	
12c	102.1 (d)	6.11 (1H, t, *J* = 2.2 Hz)	101.6 (d)	6.11 (1H, t, *J* = 2.2 Hz)

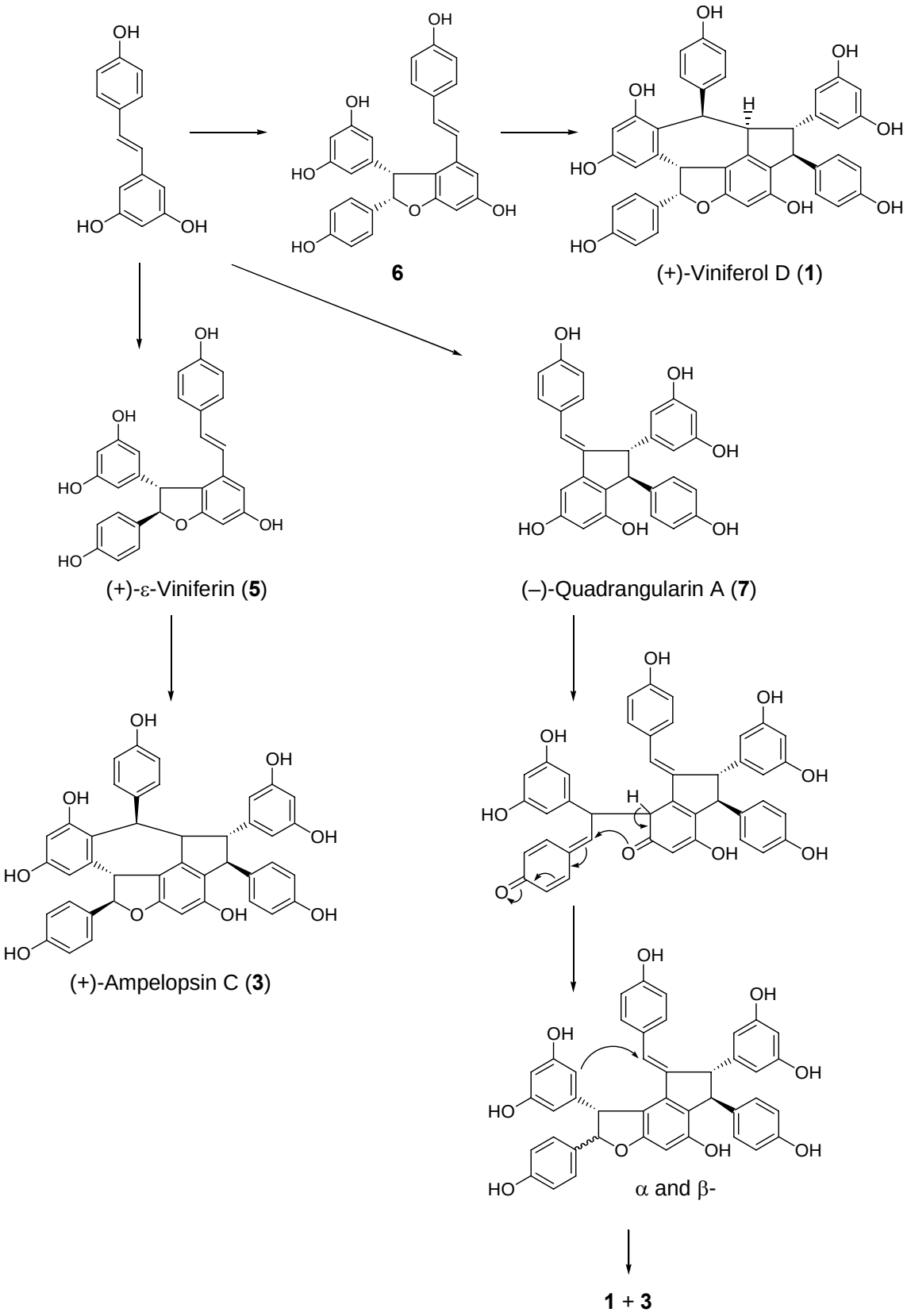


Figure 1. Biogenetic Pathways of (+)-Viniferol D (**1**) and (+)-Ampelopsin C (**3**)

between H-8b and H-8c, and between H-8c and H-7c to be *syn*, *anti* and *anti*. All stereochemistries of **1** are the same as those of (+)-ampelopsin C (**3**) except for the position 7a. The stereochemistries of two

methine hydrogens (H-7a and H-8a) of the dihydrobenzofuran moiety were assigned by comparison of the coupling constant value. In the case of (+)-ampelopsin C (**3**), the *anti* stereochemistry was assigned by the value of J = 12.0 Hz. The value of J = 2.9 Hz suggested the stereochemistry between H-7a and H-8a of (+)-viniferol D (**1**) to be *syn*. These results were further supported by the *J*-values and the dihedral angles, in Dreiding models, J = 0.5 Hz and 73.2° between H-7b and H-8b, J = 8.5 Hz and –164.4° between H-8b and H-8c, and J = 8.1 Hz and 160.3° between H-8c and H-7c. From the evidence mentioned above, the structure of (+)-viniferol D should be represented as **1** including the stereochemistry. Namely, (+)-viniferol D is an epimer of (+)-ampelopsin C at the position 7a.

Biogenesis of (+)-viniferol D

Many oligomers of resveratrol (3,5,4´-trihydroxystilbene) have been isolated from Vitaceaeous plants.[9] From the point of view of biogenesis, (+)-ε-viniferin (**5**), a dimer of resveratrol, is a very important precursor of most of them.[9,10] (+)-Ampelopsin C (**3**) seems to be also originated from (+)-ε-viniferin (**5**) as shown in Figure 1. But (+)-viniferol D (**1**) has to be formed by a different biosynthetic pathway from that of (+)-ampelopsin C (**3**). (+)-Viniferol D (**1**) can be biosynthesized by an oxidative coupling of resveratrol and a (+)-ε-viniferin-like compound (**6**),[11] or an oxidative coupling of quadrangularin A (**7**)[10,12] and resveratrol as shown in Figure 1. The (+)-ε-viniferin-like compound (**6**)[11] having a *cis*-stereochemistry between C-7a and C-8a could be isolated from the plant of 'Kyohou' in the near future.

EXPERIMENTAL

General

UV and IR spectra were recorded on JASCO Ubest V-560 (cell length 10 mm) and FT/IR-410 spectrophotometers, respectively. Optical rotations were measured with a JASCO P-1020 polarimeter (cell length 100 mm). ^{1}H and ^{13}C NMR spectra were recorded on JEOL ALPHA-600 (^{1}H: 600 MHz and ^{13}C: 150 MHz) and JEOL ALPHA-400 (^{1}H: 400 MHz and ^{13}C: 100 MHz) spectrometers. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to the solvent signal (methanol-d_4: δ_H 3.30 and δ_C 49.0) as an internal standard. LR and HR FAB-MS were obtained with JEOL JMS HX-110 using *m*-nitrobenzyl alcohol as matrix. Analytical TLC was performed on silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd.).

Isolation of (+)-viniferol D (1)

A part of the ethyl acetate fraction (29.5 g) described in the previous paper[3] of the stem of *V. vinifera* 'Kyohou' was fractionated by medium-pressure column chromatography (MPCC) (45 x 450 mm) over silica gel (265 g) using a gradient solvent system of chloroform and methanol (20:1 to 0:1) to give thirteen fractions (F-1 to F-13). F-10 (2.4 g, chloroform–methanol = 5:1) was subjected to reversed-phase MPCC (Develosil Lop C8-45S (45 x 450 mm), Nomura Chemical Co. Ltd.) using a mixed solvent of methanol–water (55:45) (flow rate: 5.0 ml/min) give 6 fractions (F-101 to F-106). A Sephadex LH-20 column chromatography of F-102 (585 mg) using methanol, followed by preparative TLC on silica gel (Merck, 05744, 0.5 mm, 20 x 20 cm, $CHCl_3$–CH_3OH–H_2O (100:20:1)) gave (+)-viniferol D (**1**) (21.6 mg) together with (+)-viniferol C (**2**) (14.3 mg).[3]

(+)-Viniferol D (1). $[\alpha]_D$ +101.4° (*c* 0.27, MeOH); a colorless amorphous solid; UV λ_{max} (MeOH) (nm (log ε)) 284 (4.04), 227 (sh, 4.73), 201 (5.02); IR ν_{max} (KBr) 3379, 1608, 1508, 1457 cm^{-1}; ^1H NMR and ^{13}C NMR data in methanol-d_4 are shown in Table 1; HRFAB-MS: *m/z* 681.2144 $[M+H]^+$ (681.2125 calculated for $C_{42}H_{33}O_9$). ^1H NMR (acetone-d_6, 400 MHz) δ 7.23 (2H, d, *J*=8.8 Hz; H-2a, 6a), 7.03 (2H, d, *J*=8.8 Hz; H-2b, 6b), 6.77 (2H, d, *J*=8.8 Hz; H-3a, 5a), 6.74 (2H, d, *J*=8.8 Hz; H-2c, 6c), 6.60 (2H, d, *J*=8.8 Hz; H-3c, 5c), 6.58 (2H, d, *J*=8.8 Hz; H-3b, 5b), 6.55 (1H, d, *J*=2.2 Hz; H-14a), 6.42 (1H, d, *J*=2.2 Hz; H-12a), 6.41 (2H, d, *J*=2.2 Hz; H-10c,14c), 6.17 (1H, t, *J*=2.2 Hz; H-12c), 6.07 (1H, d, *J*=3.3 Hz; H-7a), 6.00 (1H, s; H-12b), 5.26 (1H, br s; H-7b), 4.39 (1H, br s; H-8a), 4.38 (1H, d, *J*=9.5 Hz; H-7c), 4.21 (1H, d, *J*=11.7 Hz; H-8b), 2.95 (1H, dd, *J*=11.7, 9.5 Hz; H-8c).

(+)-Ampelopsin C (3). ^1H NMR (acetone-d_6, 400 MHz) δ 7.28 (2H, d, *J* = 8.5 Hz; H-2a, 6a), 7.20 (2H, d, *J* = 8.8 Hz; H-2b, 6b), 7.03 (2H, d, *J* = 8.5 Hz; H-2c, 6c), 6.82 (2H, d, *J* = 8.5 Hz; H-3a, 5a), 6.75 (2H, d, *J* = 8.5 Hz; H-3c, 5c), 6.70 (2H, d, *J* = 8.8 Hz; H-3b, 5b), 6.37 (1H, d, *J* = 2.0 Hz; H-12a), 6.22 (2H, d, *J* = 2.0 Hz; H-10c,14c), 6.20 (1H, t, *J* = 2.0 Hz; H-12c), 6.18 (1H, br s; H-14a), 6.17 (1H, s; H-12b), 5.85 (1H, d, *J* = 12.0 Hz; H-7a), 5.29 (1H, d, *J* = 3.5; H-7b), 4.48 (1H, d, *J* = 12.0 Hz; H-8a), 4.26 (1H, d, *J* = 9.5 Hz; H-7c), 3.78 (1H, dd, *J* = 12.0, 9.5 Hz; H-8c), 3.67 (1H, br d, *J* = 12.0 Hz; H-8b). ^{13}C NMR (acetone-d_6, 100 MHz) δ 159.2 (s; C-11b), 158.9 (s; C-11c,13c), 158.3 (s; C-4a), 156.4 (s; C-13a), 156.4 (s; C-4c), 155.6 (s; C-4b), 155.5 (s; C-11a), 154.4 (s; C-13b), 146.7 (s; C-9c), 143.9 (s; C-9b), 141.4 (s; C-9a), 133.2 (s; C-1b), 132.6 (s; C-1c), 130.6 (s; C-1a), 130.3 (d; C-2c, 6c), 130.0 (d; C-2b, 6b), 129.8 (d; C-2a, 6a), 124.6 (s; C-10a), 120.9 (s; C-14b), 116.0 (d; C-3a, 5a), 115.7 (d; C-3c, 5c),115.7 (s; C-10b), 115.4 (d; C-3b, 5b), 107.3 (d; C-10c,14c), 105.8 (d; C-14a), 101.6 (d; C-12a), 101.6 (d; C-12c), 96.5 (d; C-12b), 90.4 (d; C-7a), 61.9 (d; C-8c), 57.1 (d; C-7c), 52.3 (d; C-8b), 48.7 (d; C-8a), 37.4 (d; C-7b).

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