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OLIGOMERIC PROANTHOCYANIDINS POSSESSING A DOUBLY LINKED STRUCTURE FROM *PAVETTA OWARIENSIS**

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Key Word Index—*Pavetta owariensis*; Rubiaceae; proanthocyanidins; doubly linked structures; trimers; tetramers; pentamer; pavetannin B7; pavetannin B8; pavetannin C1; cinnamtannin B2; pavetannin D1.

Abstract—Pavetannins B7 and B8, two new trimeric proanthocyanidins, have been isolated from the stem bark of *Pavetta owariensis*, along with the known tetramers cinnamtannin B2 and its positional isomer, pavetannin C1, and the pentamer pavetannin D1. NMR and mass spectral data established the structure of the pavetannins as epicatechin-(4$\beta \rightarrow 8$, 2$\beta \rightarrow O \rightarrow 7$)-*ent*-epicatechin-(4$\alpha \rightarrow 8$, 2$\alpha \rightarrow O \rightarrow 7$)-*ent*-catechin, epicatechin-(4$\beta \rightarrow 8$, 2$\beta \rightarrow O \rightarrow 7$)-epicatechin-(4$\beta \rightarrow 8$, 2$\beta \rightarrow O \rightarrow 7$)-*ent*-catechin, epicatechin-(4$\beta \rightarrow 6$)-epicatechin-(4$\beta \rightarrow 8$, 2$\beta \rightarrow O \rightarrow 7$)-epicatechin-(4$\beta \rightarrow 8$)-epicatechin and epicatechin-(4$\beta \rightarrow 8$)-epicatechin-(4$\beta \rightarrow 8$, 2$\beta \rightarrow O \rightarrow 7$)-epicatechin-(4$\beta \rightarrow 8$)-epicatechin-(4$\beta \rightarrow 8$)-epicatechin, respectively. The naming of cinnamtannin B2 is revised to epicatechin-(4$\beta \rightarrow 8$)-epicatechin-(4$\beta \rightarrow 8$,2$\beta \rightarrow O \rightarrow 7$)-epicatechin-(4$\beta \rightarrow 8$)-epicatechin, according to its structural presentation.

INTRODUCTION

In previous papers, we have reported the isolation from stem bark of *Pavetta owariensis* P. Beauv. (Rubiaceae) and the characterization of a series of dimeric and trimeric proanthocyanidins possessing a doubly linked structure [1–3], with antiviral and antibacterial activity [4]. Continued chemical investigations on the proanthocyanidin fractions from *P. owariensis* have resulted in the isolation and characterization of additional oligomeric proanthocyanidins, all possessing a doubly linked structure.

RESULTS AND DISCUSSION

The acetone extract of the stem bark of *P. owariensis* was repeatedly chromatographed on Sephadex LH-20 to afford **1**–**5**. Although efforts to separate a chromatographically homogeneous mixture have hitherto failed, **1** and **2** were identified as follows. The FAB mass spectrum of the mixture indicated a $[M + H]^+$ ion at *m/z* 863, consistent with a triflavanoid structure. The 600 MHz ^{1}H NMR spectrum (recorded in CD_3OD) revealed the presence of two structurally related components in a ratio of 1 : 1.7. Owing to the unequal intensities of the ^{1}H NMR signals and based on earlier observations [2], the resonances characteristic for each molecule could be allocated with some certainty (Table 1). The ^{1}H NMR spectrum of the mixture composed of **1** and **2** displayed a series of isolated AB systems (δ 4.71, 4.56 and 4.37, 4.02, each *d*, *J* = 4.0 Hz for **1**; δ 4.72, 4.30 and 4.22, 4.15, each *d*, *J* = 4.0 Hz for **2**), associated with the heterocyclic H-3 and H-4 of ring C and F, respectively, of A-type proanthocyanidins, and also the conspicuous absence of the effects of dynamic rotational isomerism about the two interflavanyl bonds imposed by the successive additional carbon–oxygen linkage. Aromatic AB-patterns (δ 6.05 and 6.10 for **1**, δ 6.06 and 6.08 for **2**) for their respective A-rings and two sharp aromatic singlets (δ 6.12 and 6.20 for **1**, δ 6.11 and 6.13 for **2**) confirmed the trimeric constitution in each instance as already concluded from the FAB mass spectrum. The large coupling constant of H-2 (I) (δ 4.77, *J* = 8.0 Hz) reflected a relative 2,3-*trans* configuration and hence the presence of a catechin-like chain-terminating entity in **1**. Owing to the overlap of the analogous signal with the solvent peak, the magnitude of $J_{2,3}$ of ring I could not be defined for **2**. ^{13}C NMR spectroscopy provided complementary structural and stereochemical details. In the ^{13}C NMR spectrum, the signals arising from **1** and **2** were readily allocated (Table 2). A diagnostic feature of the ^{13}C resonances assignable to **1** was the presence of two ketal carbon signals at δ 102.5 and 103.6, indicating the presence of two

*Part 4 in the series 'Proanthocyanidins from stem bark of *Pavetta owariensis*'. For Part 3, see ref. [3].

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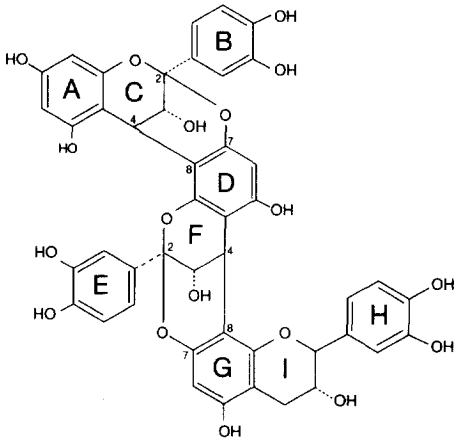
Table 1. Characteristic ^{1}H NMR spectral data for proanthocyanidins **1** and **2** (600 MHz, CD_3OD) [multiplicity and *J* (Hz)]

Ring	H	1	2
C	3	4.71 (*d*, 3.1)	4.72 (*d*, 3.1)
	4	4.56 (*d*, 3.1)	4.30 (*br s*)
F	3	4.02 (*d*, 3.1)*	4.15 (*d*, 3.1)*
	4	4.37 (*d*, 3.1)*	4.22 (*d*, 3.1)*
I	2	4.77 (*d*, 8.0)	‡
	3	4.13† (*m*)	4.05† (*m*)
	4	2.62 (1H, *dd*, 15, 7)	2.95 (2H, *m*)
		2.95 (1H, *m*)	
A	6	6.05 (*d*, 2.3)	6.06 (*d*, 2.3)
	8	6.10 (*d*, 2.3)	6.08 (*d*, 2.3)
D,G	6,8	6.12 (*s*)	6.11 (*s*)
		6.20 (*s*)	6.13 (*s*)
B,E,H	2,5,6	6.76–7.49	6.76–7.49

* Assignments may be interchanged within the same column.

† Assignments may be interchanged within the same row.

‡ Masked by solvent.



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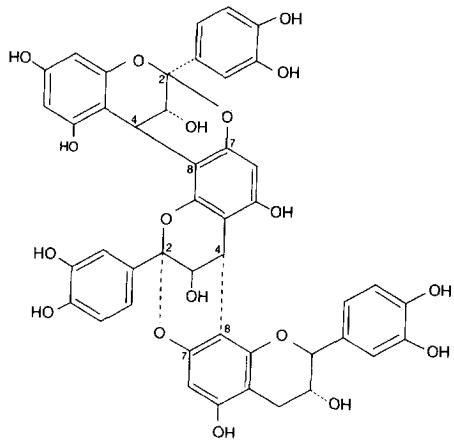

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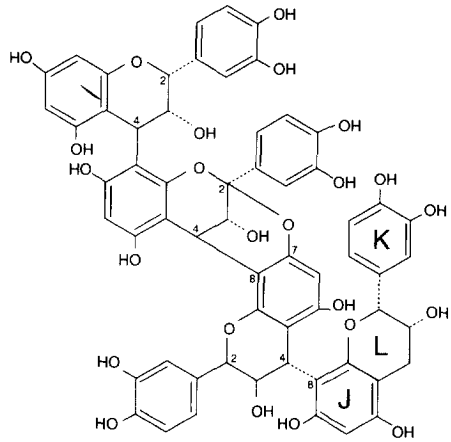

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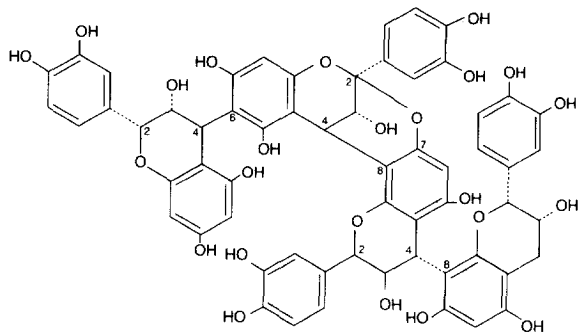

4

doubly linked flavanyl units in the molecule. These findings were in agreement with the ^{1}H NMR analysis. Further, the ^{13}C NMR signal patterns arising from the heterocyclic I-ring (81.4, 68.1 and 67.1) were reminiscent of those of the lower terminal *ent*-catechin unit of pavetannin A1 [*ent*-epicatechin-(4$\alpha \rightarrow$ 8, 2$\alpha \rightarrow O \rightarrow$ 7)-*ent*-catechin] (81.8, 68.1 and 66.9) [1, 3], suggesting that the chain-terminating GHI-unit possessed a similar A-type structure. The ^{13}C resonance of C-2(I) (δ 81.4) of **1** also suggested that an *ent*-epicatechin unit represented the middle DEF flavanyl constituent, since the analogous C-2 signal of an *ent*-catechin terminal unit attached to an epicatechin extension-unit was observed to resonate downfield at *ca* δ 83.5, as evident from proanthocyanidin A-4 [1] and aesculitannin D [5]. The chemical shift of the remaining C-3(C) carbon signal at 67.8, being close to that of epicatechin (67.4), indicated an 'upper' epicatechin unit [1]. The interflavanoid linkage between the three units of **1** was assumed to be C-4/C-8 based on the

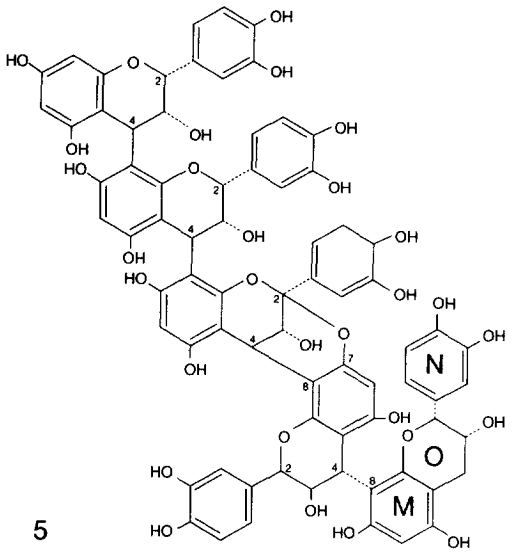

Table 2. Characteristic ^{13}C NMR spectral data for proanthocyanidins **1** and **2** (150 MHz, CD_3OD)

Ring no.	Carbon no.	1	2
C	2	103.6	103.7
	3	67.8	67.7
	4	30.4	30.7
F	2	102.5	103.1
	3	67.1	67.6
	4	29.6	29.0
I	2	81.4	83.9
	3	68.1	68.3
	4	29.8	30.0
A	6	98.3	98.5
	8	97.8	97.9
D	6	96.3	96.4
	8	108.9	107.8
G	6	96.3	96.4
	8	106.0	105.6

unsubstituted phloroglucinol ring carbons C-6(A), C-8(A) and C-6(D) at 98.3, 97.8, and 96.3, respectively, being close to those of pavetannin B6 [2, 3]. Upon consideration of the above results, **1**, designated as pavetannin B7, was characterized as epicatechin-(4$\beta \rightarrow$ 8, 2$\beta \rightarrow O \rightarrow$ 7)-*ent*-epicatechin-(4$\alpha \rightarrow$ 8, 2$\alpha \rightarrow O \rightarrow$ 7)-*ent*-catechin. This chemical name is in agreement with the consistent use of the α/β notation, as discussed in our note on the nomenclature of oligoflavonoids with an A-type unit [6].

Following ^{1}H NMR analysis, the close structural similarity between **1** and **2** was readily evident, also reflected in the ^{13}C NMR spectrum, the only notable differences being the chemical shifts of the heterocyclic protons and carbons (Tables 1 and 2). Again, the ^{13}C NMR spectrum revealed the presence of two ketal carbons at δ 103.7 and 103.1 ppm, consistent with the presence of two doubly linked flavanyl units. The chemical shifts of the signals at δ 83.9, 68.3 and 67.6 attributable to C-2(I), C-3(I) and C-3(F), respectively, were in excellent agreement with those of proanthocyanidin A-4 [epicatechin-(4$\beta \rightarrow$ 8, 2$\beta \rightarrow O \rightarrow$ 7)-*ent*-catechin] (δ 83.9, 68.5 and 67.5, respectively) [1], suggesting the constitution of proanthocyanidin A-4 as the lower biflavanoid unit. Attachment of epicatechin to a 'lower' terminal *ent*-catechin entity clearly followed from the diagnostic downfield position of C-2(I) at δ 83.9 as discussed above, also providing evidence for (4,8)-coupling between the 'lower' and 'middle' flavanyl units. The carbon signal of C-3(C) at δ 67.7 was close to **1** and other A-type proanthocyanidins with epicatechin chain-terminating constituents [2], giving support for an 'upper' unit with 2,3-*cis*-stereochemistry. The small difference in chemical shift for C-3(C) in **1** and **2** ($\Delta\delta$ 0.1 ppm) again suggested the presence of an epicatechin 'upper' unit in **2**. Conjecture regarding the C-4/C-8 interflavanoid bonds of **2** followed tentatively from the general resemblance of the NMR data of **2** and **1** (Tables 1 and 2). Thus, the trimer **2**, designated as pavetannin B8, was identified as epicatechin-(4$\beta \rightarrow$ 8, 2$\beta \rightarrow O \rightarrow$ 7)-epicatechin-(4β-8, 2$\beta \rightarrow O \rightarrow$ 7)-*ent*-catechin. Pavetannin B7 and B8 represent rather uncommon trimeric proanthocyanidins in that they have two doubly bonded interflavanoid linkages in each molecule, representatives of this class of natural products being to date aesculitannins C and D only [5].

On TLC, **3** showed the same R_f value (0.10) as an authentic sample of cinnamtannin B2. In the FAB mass spectrum, a $[M + H]^+$ ion was detected at m/z 1153, which suggested a tetraflavanoid moiety. The ^{1}H and ^{13}C NMR spectra of **3** (recorded in CD_3OD at 200 and 50 MHz, respectively) were superimposable with those of authentic cinnamtannin B2. Accordingly, **3** was identified as cinnamtannin B2 or epicatechin-(4$\beta \rightarrow$ 8)-epicatechin-(4$\beta \rightarrow$ 8, 2$\beta \rightarrow O \rightarrow$ 7)-epicatechin-(4$\beta \rightarrow$ 8)-epicatechin. It should be noted that originally the full chemical name of cinnamtannin B2 was given as epicatechin-(4$\beta \rightarrow$ 8)-epicatechin-(4$\beta \rightarrow$ 8, 2$\beta \rightarrow O \rightarrow$ 7)-epicatechin-(4$\alpha \rightarrow$ 8)-epicatechin [7]. However, the stereochemistry of the linkage between the two 'lower' units should be denoted as β, since the third unit of the tetramer is not graphically depicted in its normal conventional orientation, but has been rotated over 180° [6].

The FAB mass spectrum of **4** indicated a $[M + H]^+$ ion at m/z 1153, indicating a tetrameric structure. The ^{1}H NMR spectrum of **4**, recorded at room temperature in CD_3OD, was exceedingly complex due to the effects of dynamic rotational isomerism, but similar to that of cinnamtannin B2 (**3**). In the ^{13}C NMR spectrum of **4**, which was reminiscent of that of **3**, the presence of four flavanyl units was indicated by the C-3 signals at δ 72.4, 71.3, 67.4 and 66.8, and the C-4 signals at δ 38.3, 37.7, 29.7 and 28.9 (major rotameric signals). The carbon signals at δ 80.2, 78.7 and 76.7 were consistent with the C-2 resonances of epicatechin moieties. The presence of an A-type proanthocyanidin in the tetrameric chain with epicatechin constituent flavanyl units was evident from the

characteristic ketal carbon resonance at δ 104.9. Moreover, **4** showed a strong positive Cotton effect, indicating a β-orientation of the interflavanyl linkages as depicted in its formulation. Owing to insufficient sample quantity, we were unable to embark on a protocol of chemical degradation to obtain additional structural information via partial cleavage products. However, the rather unusual chemical shift of the broadened ^{1}H NMR signal at δ 5.65 attributable to H-2(C) and of the H-2(I) signal, assumed at δ 4.88, being obscured by the solvent signal, implied steric interactions with aromatic B-rings of flavanyl units. This may reasonably be explained on the basis of an 'upper' (4,6)-interflavanyl linkage, as evident from Dreiding models. Independent support regarding the (4,6)-coupling was available from deshielding of C-4(C) [+ 0.6 ppm] relative to the corresponding signal in **3** and the ^{13}C resonance of C-6(D) [δ 108.8], in favour of a substituted carbon. Thus, **4**, designated as pavetannin C1, was identified as epicatechin-(4β → 6)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin, recently characterized by similar observations derived from partial degradation products [7]. The naming of **4** is similarly revised as **3**, consistent with its graphical representation [6].

In FAB MS, **5** showed a $[M + H]^+$ ion at *m/z* 1441, which was characteristic for a pentameric structure. The ^{1}H and ^{13}C NMR spectra were poorly resolved. However, in the ^{13}C NMR spectrum the C-2 signals at δ 80.2, 78.8, 77.8 and 76.6, the C-3 signals at δ 72.6, 72.6, 71.4, 67.1 and 67.1, and the ketal carbon at δ 104.9 could be clearly observed. These features suggested a pentameric procyanidin, consisting only of epicatechin units, and possessing an A-type structural element. In addition, **5** showed the same low R_f value (0.02) as an authentic pentameric specimen. Although degradative studies were precluded by the lack of material, the chemical shifts of the heterocyclic ring carbons in **5**, being close to those reported by Nonaka *et al.* [7] for a pentameric procyanidin with an A-type unit, suggested **5** to possess the same constitution. Upon consideration of these results, **5**, designated as pavetannin D1, was tentatively identified as epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin, taking into account the consistent use of the α/β nomenclature as discussed above. Formation of the pentamer **5** may be attributed to coupling of the 2,3-*cis*-flavan-3,4-diol as potential precursor with cinnamatannin B2 (**3**), co-occurring in the same plant material.

EXPERIMENTAL

General. General experimental procedures have been described in earlier publications [1–3].

Isolation. The isolation of proanthocyanidin frs from *P. owariensis* has been published earlier [1]. Briefly, **1**–**5** were obtained from polar, enriched proanthocyanidin frs by repetitive CC on Sephadex LH-20 with EtOH.

Compounds **1** [pavetannin B7, epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-epicatechin-(4α → 8, 2α → *O* → 7)-*ent*-catechin] and **2** [pavetannin B8, epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8, 2β → *O* → 7)-*ent*-catechin] were obtained as an inseparable mixt. in a yield of *ca* 0.003%. On TLC (solvent A: EtOAc–HCO_2H–HOAc–H_2O; 140:2:1:59; upper phase), both polyphenols migrated as a single band with R_f 0.32. FAB MS: *m/z* 863 $[M + H]^+$. ^{1}H and ^{13}C NMR: Tables 1 and 2, respectively.

Compound **3** [Cinnamtannin B2, epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin] showed an R_f value of 0.10 on TLC (solvent A), and it responded positively to the vanillin–sulpuric acid and anisaldehyde–sulpuric acid reagents. It was obtained in a yield of 0.010%. FAB MS: *m/z* 1153 $[M + H]^+$. ^{1}H NMR (200 MHz, CD_3OD): δ 2.83 [2H, *m*, H-4(L)], 3.62–5.65 [heterocyclic protons], 5.98–6.30 [H-6, H-8 (A, D, G and J)], 6.65–7.31 [H-2′, H-5′, H-6′ (B, E, H, K)]. ^{13}C NMR: see ref. [7].

Compound **4** [pavetannin C1, epicatechin-(4β → 6)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin] showed an R_f value of 0.08 on TLC (solvent A), and it displayed a similar colour response to the above spray reagents as **3**. It was isolated in a yield of 0.025%. FAB MS: *m/z* 1153 $[M + H]^+$. ^{1}H NMR (200 MHz, CD_3OD): δ 2.78 [2H, *m*, H-4(L)], 4.00–5.40 (heterocyclic protons), 5.65 [1H, *br s*, H-2(C)], 5.90–6.30 [H-6, H-8 (A, D, G and J)], 6.65–7.32 [H-2′, H-5′, H-6′ (B, E, H, K)]. ^{13}C NMR (50 MHz, CD_3OD): δ 28.9 [C-4(F)], 29.7 [C-4(L)], 37.7 [C-4(C)], 38.4 [C-4(I)], 66.8 [C-3(L)], 67.4 [C-3(F)], 71.3 [C-3(C)], 72.4 [C-3(I)], 76.7 [C-2(C)], 78.7 [C-2(I)], 80.2 [C-2(L)], 96.6, 98.3 [C-6(A), C-8(A), C-8(D). C-6(G), C-6(J)], 100.1 [C-4a (A, D, G, J)], 104.9 [C-2(F)], 106.9, 107.3, 107.7 [C-8(G,J), C-4a (A or D or G or J)], 108.8 [C-6(D)], 116.2, 116.7 [C-5(B, E, H, K)], 119.3, 119.9, 120.7, 121.4 [C-6(B, E, H, K)], 131.6, 131.6, 132.3. 132.8 [C-1 (B, E, H, K)], 145.8, 145.8, 145.8, 146.3, 146.3, 146.3, 146.7, 146.7 [C-3 and C-4 (B, E, H, K)], 150.3, 154.2, 155.5, 155.5, 155.9, 156.7, 156.7, 157.8, 157.8, 157.8, 159.2 (C-5, C-7 and C-8a (A,D,G,J)]. CD $[\theta]_{236}$: + 44 849, $[\theta]_{260}$: − 6156.

Compound **5** [pavetannin D1, epicatechin-(4β → 8)-epicatechin-(4β → 8, 2β → *O* → 7)-epicatechin-(4β → 8)-epicatechin-(4β → 8)-epicatechin] was obtained from proanthocyanidin frs by repetitive CC on Sephadex LH-20 using MeOH–Me_2CO (8:2) as eluant, in a yield of 0.013%. On TLC it showed the same R_f-value (0.02, solvent A) as an authentic sample of a pentameric procyanidin. FAB MS: *m/z* 1441 $[M + H]^+$. ^{1}H NMR (200 MHz, CD_3OD): δ 2.83 [C-4(O)], 3.87–5.25 [C, F, I, L and O ring protons), 5.70–6.12 [A, D, H, J and M-ring protons], 6.83–7.30 [B, E, G, K and N-ring protons]. ^{13}C NMR (50 MHz, CD_3OD): δ 28.9 [C-4(O)], 29.7 [C-4(I)], 37.7 [C-4(C, F)], 38.6 [C-4(L)], 67.1 [C-3(I,O)], 71.4 [C-3(C)], 72.6 [C-3(F,L)], 80.2, 78.8, 77.8, 76.6 [C-2 (O, L, F, C, respectively)], 104.9 [C-2(L)]. CD $[\theta]_{251}$ + 20 503, $[\theta]_{252}$ + 20 980, $[\theta]_{264}$ − 6437.

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