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RAPID COMMUNICATION

Novel compounds isolated from health food products containing beni-koji (red yeast rice) with adverse event reports

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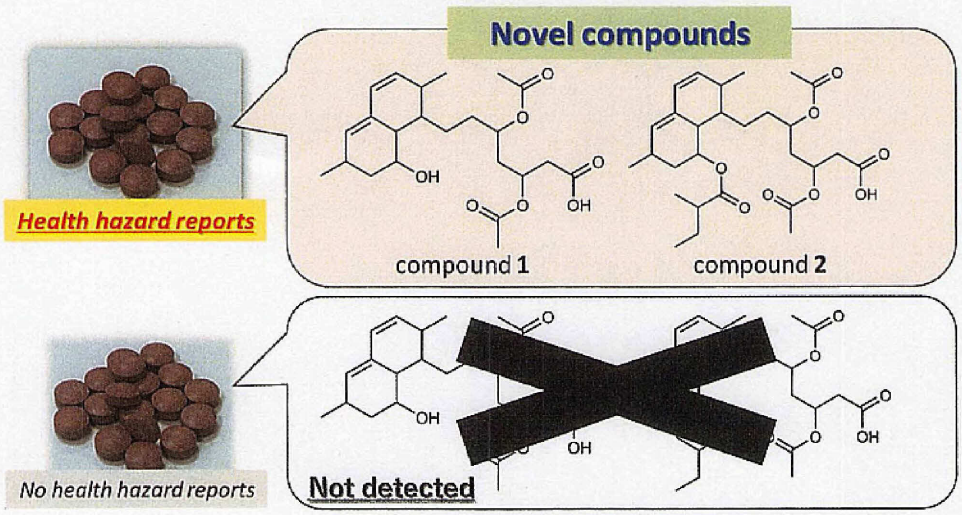
Abstract

Recently, health hazards, such as kidney damage, have been reported owing to the ingestion of a health food product, so-called "foods with functional claims (FFC)'', containing beni-koji (red yeast rice). Although not an expected compound in the FFC, the detection of puberulic acid has also been reported. Further investigations of these health food products, such as the identification of other unintended compounds and clarifying the health impacts of puberulic acid, are required. To clarify the causes of these health issues, we investigated the presence of unintended compounds in the FFC containing beni-koji using comprehensive instrumental analyses. Using differential analysis, novel compounds **1** and **2** were detected as unexpected components between the samples with and without adverse event reports. Although limited to the samples available for analyses in this study, both compounds **1** and **2** were detected in all the samples that also contained puberulic acid. Compounds **1** and **2**, with molecular formulas of $C_{23}H_{34}O_7$ and $C_{28}H_{42}O_8$, respectively, may be lovastatin derivatives. Their structures were confirmed using NMR analyses and are novel natural compounds. For definitive confirmation, we are in the process of synthesizing compounds **1** and **2** from lovastatin. The route of contamination of these compounds are currently under investigation. The findings of this study could be used to address the growing health hazards associated with health food products.

Graphical abstract



Keywords Health hazard · Beni-koji · *Monascus pilosus* · Lovastatin derivatives · Health food product · Comprehensive analysis

Seiji Tanaka, Naoko Masumoto, and Takuya Makino have contributed equally to this work.

Extended author information available on the last page of the article

Introduction

In late March 2024, some cases of health hazard reports, including kidney damage, were publicized as a result of ingesting a health food product so-called "*kinosei hyoji shokuhin* (foods with functional claims, FFC)" containing beni-koji (*Monascus pilosus*) [1]. Lovastatin (monacolin K (lactone form)) is the functional component of the FFC because it is expected to reduce blood cholesterol. Although not an intended compound in these products, puberulic acid was also detected. The presence of unintended compounds other than puberulic acid in the FFC and the contribution of puberulic acid to health hazards remain unsolved.

In this study, as a first step in clarifying the causes of these health hazards, we investigated the presence of unintended compounds in the FFC containing beni-koji using comprehensive instrumental analyses.

Results and discussion

The FFC was tablet-type products and made from "raw materials" and excipients. In this study, two raw material samples, lots A and B, were analyzed and compared. Lot A was an ingredient of the FFC with health hazard reports, while lot B was not. Sample solutions were prepared by extracting each lot with 75% (vol%) methanol, followed by ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS, Thermo), LC–MS (Shimadzu), supercritical fluid chromatography-HRMS (SFC-HRMS, Shimadzu), and GC–MS (Shimadzu) analyses.

Differential analysis of the UHPLC-HRMS data detected compounds **1** and **2** as the components in lot A. Compound **1** was eluted at 20.2 min using UHPLC-HRMS, and its molecular formula was $C_{23}H_{34}O_7$ (*m/z*: 421.2217 ($[M\text{-}H]^-$) and 423.2374 ($[M+H]^+$)). Compound **2** was eluted at 28.1 min, and its molecular formula was $C_{28}H_{42}O_8$ (*m/z*: 505.2805 ($[M\text{-}H]^-$), 507.2981 ($[M+H]^+$), and 524.3225 ($[M+NH_4]^+$) (Fig. S1).

Obtained as far back as possible (three years), 29 raw material samples including lots A and B were investigated to determine whether compounds **1** and **2** were present. The analyses revealed that both compounds **1** and **2** were detected in seven raw material samples that were manufactured during a specific period (approximately six months). Compounds **1** and **2** were not detected in samples produced outside of that specific period. Moreover, two FFC samples with health hazard reports and one FFC sample with no report were analyzed, revealing that compounds **1** and **2** were detected in only products with health hazard reports. Although limited to the samples available for analyses in this study, both compounds **1** and **2** were detected in all the samples that also contained puberulic acid.

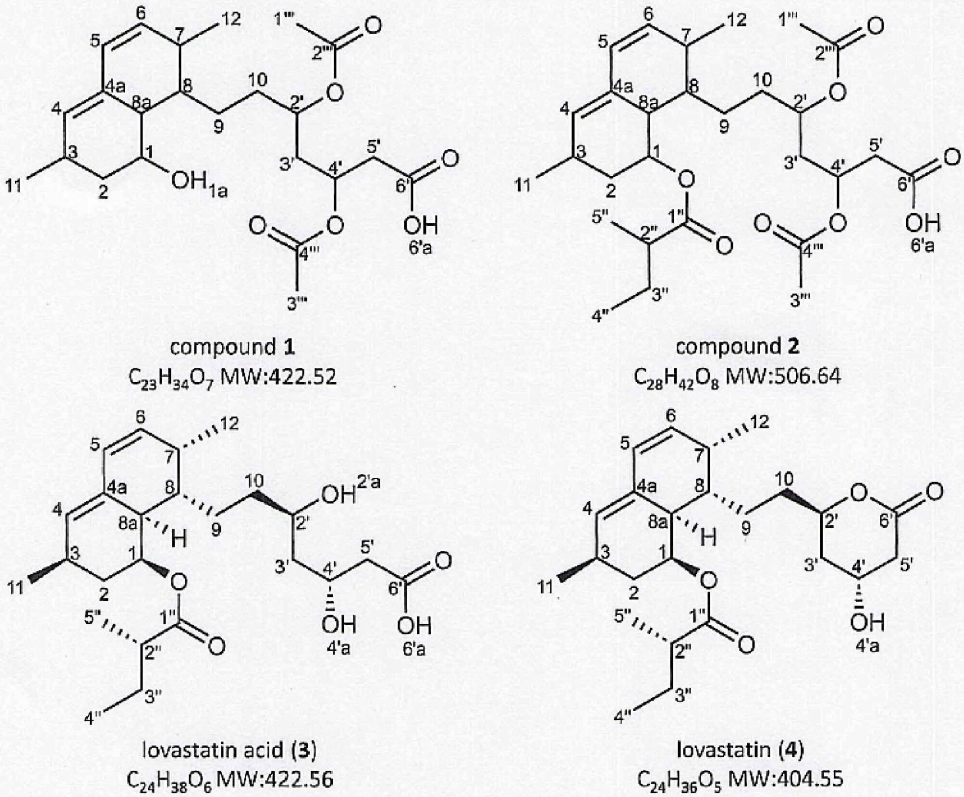


Fig. 1 Chemical structures of compounds **1** and **2**, lovastatin acid (**3**), and lovastatin (**4**)

Table 1 ^{1}H- and ^{13}C-NMR signal assignments for compound **1**, compound **2**, lovastatin acid (**3**), and lovastatin (**4**) (δ in ppm, *J* in Hz)[a]

Assignment	Compound **1**		Compound **2**		Lovastatin acid (**3**)		Lovastatin (**4**)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
CH (1)	4.03 (1H, q, 3.2)	62.8	5.21 (1H, q, 3.2)	67.4	5.19 (1H, q, 3.2)	67.5	5.23 (1H, q, 3.3)	67.5
CH_2 (2)	1.70 (1H, m) 1.74 (1H, m)	35.7	1.80 (1H, m) 1.94 (1H[b])	32.0	1.84 (1H, dd, 14.9, 3.8) 1.94 (1H[c])	31.8	1.83 (1H, dd, 15.0, 3.6) 1.94 (1H, ddd, 14.8, 8.1, 2.4)	31.9
CH (3)	2.29 (1H, m)	27.6	2.37 (1H, m)	26.9	2.40 (1H[c])	26.9	2.40 (1H, m)	26.9
CH (4)	5.38 (1H, t, 3.4)	129.3	5.48 (1H, t, 3.7)	129.2	5.48 (1H, t, 3.3)	129.0	5.50 (1H, t, 3.5)	129.2
C (4a)		131.9		131.5		131.8		131.5
CH (5)	5.87 (1H, d, 9.6)	128.9	5.94 (1H, d, 9.7)	128.2	5.93 (1H, d, 9.7)	128.2	5.95 (1H, d, 9.6)	128.2
CH (6)	5.71 (1H, dd, 9.6, 6.0)	132.6	5.77 (1H, dd, 9.7, 6.0)	133.1	5.77 (1H, dd, 9.7, 5.8)	133.3	5.78 (1H, dd, 9.7, 6.0)	133.0
CH (7)	2.25 (1H, m)	30.2	2.30 (1H, m)	30.0	2.32 (1H[c])	30.2	2.34 (1H, m)	30.1
CH (8)	1.68 (1H, m)	35.3	1.52 (1H, m)	36.0	1.57 (1H[c])	36.5	1.60 (1H, m)	36.0
CH (8a)	1.94 (1H[b])	37.9	2.24 (1H[b])	36.4	2.24 (1H, m)	36.3	2.31 (1H, m)	36.4
CH_2 (9)	1.01 (2H, m)	23.0	1.10 (2H, m)	22.6	1.25 (2H[c])	24.2	1.27 (1H, m) 1.37 (1H, m)	23.6
CH_2 (10)	1.40 (1H, m) 1.56 (1H, m)	30.7	1.27 (1H, m) 1.57 (1H, m)	30.4	1.38 (1H[c]) 1.08 (1H, m)	34.4	1.26 (1H, m) 1.74 (1H[b])	32.4
CH_3 (11)	1.13 (3H, d, 7.3)	23.1	1.01 (3H, d, 7.3)	22.6	1.01 (3H[c])	22.5	1.02 (3H, d, 7.0)	22.6
CH_3 (12)	0.79 (3H, d, 7.0)	13.8	0.80 (3H, d, 7.0)	13.6	0.83 (3H[c])	13.6	0.85 (3H, d, 7.0)	13.6
CH (2')	4.81 (1H, m)	71.2	4.76 (1H, m)	71.0	3.46 (1H, tt, 8.2, 4.3)	68.7	4.47 (1H, m)	75.8
CH_2 (3')	1.82 (2H, m)	37.9	1.80 (2H, m)	37.6	1.39 (1H[c]) 1.49 (1H, m)	44.5	1.62 (1H, m) 1.76 (1H, dtd, 14.0, 3.6, 2.0)	35.3
CH (4')	5.09 (1H, m)	68.9	5.06 (1H, m)	69.2	3.95 (1H, m)	65.9	4.10 (1H, sext, 3.6)	61.2
CH_2 (5')	3.50 (2H, m)	69.8	3.51 (2H, m)	69.8	2.19 (1H, dd, 14.9, 8.1) 2.33 (1H[c])	42.5	2.38 (1H, m) 2.61 (1H, dd, 17.3, 4.5)	38.5
C (6')		165.3		165.3		173.0		170.2
OH (6'a)	8.39 (brs)		8.43 (brs)		8.14 (brs)			
C (1'')				175.4		175.5		175.5
CH (2'')			2.25 (1H, m)	40.7	2.28 (1H[c])	40.7	2.30 (1H, m)	40.7
CH_2 (3'')			1.38 (1H, ddd, 13.9, 8.2, 6.3) 1.52 (1H, m)	26.4	1.55 (1H[c]) 1.38 (1H[c])	26.3	1.39 (1H, m) 1.56 (1H, dt, 13.4, 7.4)	26.3
CH_3 (4'')			0.80 (3H, t, 7.3)	11.3	0.82 (3H[c])	11.3	0.83 (3H, t, 7.3)	11.3
CH_3 (5'')			1.01 (3H, d, 6.9)	15.9	1.02 (3H[c])	16.0	1.02 (3H, d, 6.8)	16.0
CH_3 (1''')	1.98 (3H, s)	21.0	1.96 (3H, s)	20.9				
C (2''')		170.0		169.8				
CH_3 (3''')	1.94 (3H, s)	21.0	1.92 (3H, s)	21.0				
C (4''')		169.7		169.6				

[a]Recorded in 800 MHz (^{1}H)/201 MHz (^{13}C) in DMSO-d_6 at 25 °C. Chemical shift scales were referenced to the DMSO signals in the deuterated solvent at 2.50 (^{1}H) and 39.52 (^{13}C) ppm

[b]Coupling information overlapped with another signal derived from itself was not described

[c]Coupling information for the signals of lovastatin acid (**3**) overlapped with signals derived from lovastatin (**4**) was not described. The spectra of the lovastatin acid (**3**) reagent used in this study contained signals derived from lovastatin (**4**) as minor signals

Compounds **1** and **2** were isolated from the samples, and UHPLC-HRMS, GC–MS, and NMR analyses were conducted to identify their structures. The results of ^{15}N-NMR, ^{19}F-NMR, and ^{31}P-NMR analyses indicated that nitrogen, fluorine, and phosphorus atoms did not exist in compounds **1** and **2**. The isotope pattern results of the UHPLC-HRMS analysis indicated that sulfur atoms were also not present in compounds **1** and **2**. Furthermore, the MS/MS spectra of UHPLC-HRMS and EI-MS spectra of GC–MS revealed that compounds **1** and **2** have a similar substructure to that of lovastatin acid (**3**) and lovastatin (**4**) (Fig. S2). As references for identification, lovastatin acid (**3**) and lovastatin (**4**)

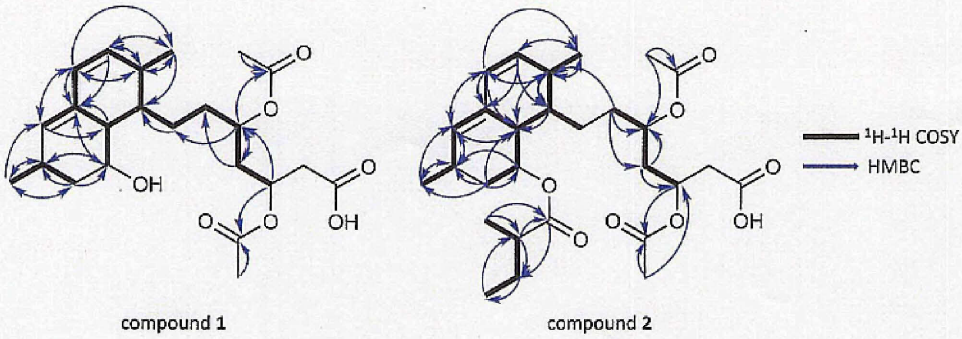


Fig. 2 Selected 2D NMR correlations for compounds **1** and **2**

(Fig. 1) were analyzed using NMR spectroscopy, as well as compounds **1** and **2**.

Compounds **1** and **2** were dissolved in dimethyl sulfoxide (DMSO)-d_6 and analyzed using NMR (^{1}H-NMR, ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT), ^{1}H-^{1}H COSY, HMQC, HMBC, and ^{1}H-^{13}C heteronuclear 2-bond correlation (H2BC)). ^{1}H- and ^{13}C-NMR data of compounds **1** and **2** in DMSO-d_6 are listed in Table 1, and spectrum data are shown in the supplementary materials (Fig. S3–S26). The ^{1}H-^{1}H COSY and HMBC correlations of compounds **1** and **2** are shown in Fig. 2. In the ^{1}H- and ^{13}C-NMR data of compounds **1** and **2**, specific signals appeared that were not detected in lovastatin acid (**3**) and lovastatin (**4**). These signals were derived from the two acetyl groups detected at δ_C/δ_H: 21.0/1.98 and 21.0/1.94 ppm for compound **1** and δ_C/δ_H: 20.9/1.96 and 21.0/1.92 ppm for compound **2**. In addition, the structure of compound **2** was probably a derivative of compound **1**, whose side chain that was originally attached to C1 was removed. Consequently, the structures of the novel compounds **1** and **2** were presumed, as shown in Fig. 1.

To confirm our results and determine the steric configuration, we are currently synthesizing compounds **1** and **2** from lovastatin (**4**) for NMR and UHPLC-HRMS analyses to determine whether they are consistent with those of compounds **1** and **2** isolated from the samples, respectively. The route of contamination of these compounds are also currently under investigation.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11418-024-01827-w.

Author contributions Seiji Tanaka and Naoko Masumoto were involved in data acquisition, analysis, interpretation, and manuscript preparation. Takuya Makino was involved in data acquisition, analysis, interpretation, and compound isolation. Yuji Matsushima was involved in sample collection and data interpretation. Toshio Morikawa was involved in data interpretation, compound isolation, and support for structure elucidation. Michiho Ito was involved in data interpretation and supervised the study. All authors critically revised the report, commented on drafts of the manuscript, and approved the final version.

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Declarations

Conflict of interest The authors Takuya Makino and Yuji Matsushima are employees of Kobayashi Pharmaceutical Co., Ltd. The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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