JOURNAL OF NATURAL PRODUCTS

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Article

Pennelliisides A–C, 2,3,4-Trisubstituted Acyl Glucoses Isolated from *Solanum pennellii*

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Cite This: https://dx.doi.org/10.1021/acs.jnatprod.9b01234

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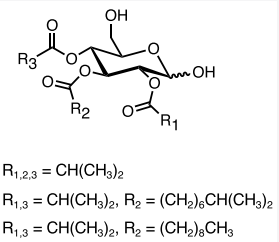
ABSTRACT: *Solanum* species accumulate a variety of secondary metabolites in their trichomes, and it is well known that acyl sugars are specialized metabolites secreted by the trichomes. However, very few reports provide detailed information on the chemical structure of polyacylated glucose derivatives, due to the α and β isomerization that can occur at the C-1 position. In this study, a strategy was established to isolate polyacylated glucose derivatives. According to the developed strategy, hydroxy groups were derivatized to a benzyloxy group using TriBOT. After isolation of the compounds in pure form and deprotection of the benzyloxy group, the chemical structures of pennelliisides A–C were determined as 2,3,4-*O*-triisobutyryl-D-glucose, 3-*O*-(8-methylnonanoyl)-2,4-*O*-diisobutyryl-D-glucose, and 3-*O*-decanoyl-2,4-*O*-diisobutyryl-D-glucose, respectively. Structural elucidation was performed using spectroscopic techniques, including 1D and 2D NMR, FD-MS, and GC-MS. It was also found that the fatty acid moiety contributes to the allelopathic properties of the isolated compounds.



1 $R_{1,2,3} = CH(CH_3)_2$

2 $R_{1,3} = CH(CH_3)_2$, $R_2 = (CH_2)_6CH(CH_3)_2$

3 $R_{1,3} = CH(CH_3)_2$, $R_2 = (CH_2)_8CH_3$

Plants produce a series of specialized metabolites, some of which are known to be important for subsistence, such as for protection against pathogens and herbivory.[1,2] A tomato species, *Solanum pennellii* Correll (Solanaceae), is categorized as a wild-type tomato, for which the full genomic information was investigated by the 100 Tomato Genome Sequencing Consortium in 2014.[3] *Solanum* species accumulate a variety of secondary metabolites in their trichomes, which, in the case of *S. pennellii*, account for more than 25% of the total dry weight.[4] It is well known that acyl sugars are specialized metabolites secreted by the trichomes.[4] For *S. pennellii*, it was reported that its acyl sugars have insecticidal,[5] pest repellant,[6] and weed growth inhibitory[7] activities. Although the fruits of the plant contain toxic glycoalkaloids,[8,9] *S. pennellii* is regarded as an agriculturally useful species due to its ability to hybridize with cultivar tomatoes.[10] Recently, it was revealed that insects can modulate not only the trichome density but also the contents of allelochemicals, for which the degree of response is thought to depend on the magnitude and/or type of induction.[11] Thus, it was hypothesized that the biosynthesis of acyl sugars might be affected by fluctuations in plant hormones. One research group has studied the biosynthetic pathway of those acyl sugars.[12] However, their biosynthesis remains to be investigated, especially due to the lack of chemical structural information.

From the chemical structural viewpoint, the structures of acyl sugars can be classified into two main groups: polyacylated sucrose and polyacylated glucose.[2] However, very few reports provide detailed information on the chemical structure[13] of polyacylated glucose derivatives, due to the α and β isomerization that can occur at the C-1 position. In this study, a strategy was established to isolate the acylglucoses of *S. pennellii* as follows: (1) protection of the hydroxy group of acylglucose using a reagent to make benzyl ether derivatives, (2) isolation and evaluation of the chemical structures of the isolated derivatives, and (3) deprotection of the benzyl group to give naturally derived compounds. Following this strategy, herein the isolation and structural determination of one known compound, 2,3,4-*O*-triisobutyryl D-glucose, and two new acylglucoses, 2,4-*O*-diisobutyryl 3-*O*-(8-methylnonanoyl)-D-glucose and 3-*O*-decanoyl 2,4-*O*-diisobutyryl-D-glucose, named, in turn, pennelliisides A–C (**1**–**3**), respectively, were accomplished, and the results are presented in this report. Furthermore, the allelopathic properties of these compounds were investigated.

RESULTS AND DISCUSSION

The upper parts of *S. pennellii* (1.7 kg) were rinsed with ethanol, and the volatile components of the organic solvent were removed under reduced pressure to give an epicuticular lipophilic extract (Figure S1, Supporting Information). The crude extract was roughly purified using column chromatog-

Received: December 15, 2019

raphy, which was followed by benzylation to give a crude extract containing benzyl derivatives of acylglucose. The benzyl derivatives were purified to give compounds **4** (47 mg), **5** (33 mg), and **6** (17 mg).

Compound **4** was isolated as a colorless oil. The molecular formula, $C_{32}H_{42}O_9$, was deduced from the HRFDMS data (m/z 570.2823 $[M]^+$, calcd 570.2829) (Figure S2, Supporting Information), and the index of hydrogen deficiency was calculated to be 12. The ^{1}H NMR resonances (Figure S3 and Table S1, Supporting Information) and the correlations observed in the COSY spectrum (Figure 2 and Figure S4, Supporting Information) between the signals at δ_H 4.36 (H-1, $J_{1,2}$ = 6.3 Hz), 5.45 (H-2, $J_{2,3}$ = 8.6 Hz), 5.43 (H-3, $J_{3,4}$ = 9.3 Hz), 5.29 (H-4, $J_{4,5}$ = 9.3 Hz), 3.39 (H-5), and 3.47 (H-6), together with their coupling constants (Figure 2A), suggested that the basic skeleton of **4** is a glucopyranose backbone, as supported by the HMBC correlations (Figures 2B and S5, Supporting Information). The NOESY correlations shown in Figures 2C and S6 (Supporting Information) showing H-1/H-3/H-5 cross-peaks substantiated this determination, and the δ_C 99.5 resonance in the ^{13}C NMR spectrum (Figure S7 and Table S1, Supporting Information) indicated a β anomeric structure. The HMBC correlations (Figures 2 and S5, Supporting Information) of H-2 (δ_H 5.45)/C-A-1 (δ_C 174.4), H-3 (δ_H 5.43)/C-B-1 (δ_C 175.5), and H-4 (δ_H 5.29)/C-C-1 (δ_C 174.6) were indicative of the linkage of the fatty acid moiety with the basic glucose structure. Furthermore, the HMBC correlations (Figures 2 and S5, Supporting Information) of H-A-2 (δ_H 2.41, methine proton)/C-A-1 (δ_C 174.4), H-B-2 (δ_H 2.39, methine proton)/C-B-1 (δ_C 175.5), and H-C-2 (δ_H 2.30, methine proton)/C-C-1 (δ_C 174.6), together with the COSY correlations (Figures 2 and S4, Supporting Information) of δ_H 1.07 (H-A-3 and A4) to 2.41 (H-A-2), δ_H 1.06 (H-B-3 and B-4) to 2.39 (H-B-2), and δ_H 1.01 (H-C-3) and 0.97 (H−C4) to 2.30 (H−C-2), suggested that three isobutyryl ester moieties are attached to the basic glucose structure. In addition, the HMBC correlations between the phenylmethylene protons of δ_H 4.74/4.73 (H-1a′ and -1b′) and anomeric carbon C-1 (δ_C 99.5) and between the phenylmethylene protons of δ_H 4.33/4.30 (H-1a″ and 1b″) and C-6 (δ_C 69.2) indicated that two substituted benzyloxy groups are attached at the C-1 and C-6 positions. Therefore, it was determined that the structure of **4** is 1,6-*O*-dibenzyl-2,3,4-*O*-triisobutyryl-β-D-glucose. Analysis of the ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, and HMBC spectroscopic data gave the total ^{1}H and ^{13}C NMR assignments for **4**, as listed in Table S1 (Supporting Information).

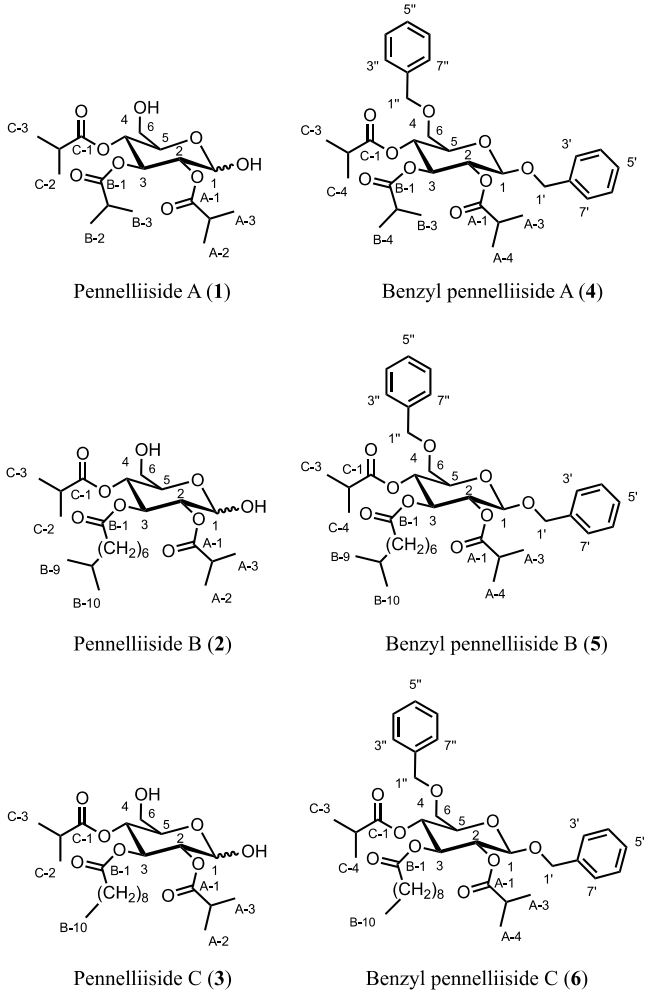


Figure 1. Structures of pennelliisides A−C and their derivatives.

Compound **5** was purified as a colorless oil. The molecular formula, $C_{38}H_{54}O_9$, was determined from the HRFDMS data (m/z 654.3750 $[M]^+$, calcd 654.3767) (Figure S9, Supporting Information) with the index of hydrogen deficiency again calculated to be 12. The ^{1}H NMR resonances (Figure S10 and Table S2, Supporting Information) and the correlations observed in the COSY spectrum (Figures 3 and S11, Supporting Information) between the signals at δ_H 4.39 (H-1, $J_{1,2}$ = 7.7 Hz), 5.46 (H-2, $J_{2,3}$ = 9.0 Hz), 5.49 (H-3, $J_{3,4}$ = 9.2 Hz), 5.31 (H-4, $J_{4,5}$ = 9.1 Hz), 3.43 (H-5), and 3.48 (H-6b), together with their coupling constants, suggested that the basic skeleton of **5** is also a glucopyranose backbone and again was supported by the HMBC correlations shown in Figures 3 and S12 (Supporting Information). The NOESY correlations in Figures 3 and S13 (Supporting Information) showing H-1/H-3/H-5 cross-peaks substantiated this determination, and the δ_C 99.4 resonance in the ^{13}C NMR spectrum (Figure S14, Table S2, Supporting Information) indicated a β anomeric structure. The correlations in the HMBC spectrum (Figures 3 and S12, Supporting Information) of H-2 (δ_H 5.46)/C-A-1(δ_C 174.5), H-3 (δ_H 5.49)/C-B-1 (δ_C 172.3), and H-4 (δ_H 5.31)/C-C-1(δ_C 174.6) were indicative of the linkage of the fatty acid moiety with the basic glucose structure. Furthermore, the correlations in the HMBC spectrum (Figures 3 and S12, Supporting Information) of H-A-2 (δ_H 2.45, methine proton)/C-A-1 (δ_C 174.5) and H-C-2 (δ_H 2.34, methine proton)/C-C-1(δ_C 174.6) together with the correlations in the COSY spectrum (Figures 3 and S11, Supporting Information) of δ_H 1.12 (H-A-3 and H-A-4) to 2.45 (H-A-2) and δ_H 1.05 (H−C-3) and 1.00 (H−C-4) to 2.34 (H−C-2) suggested that two isobutyryl ester moieties are attached to the basic glucose structure. In addition, the correlations in the COSY spectrum (Figures 3 and S11, Supporting Information) of δ_H 0.88 (H-B-9 and B-10, each 3H) and δ_H 1.47 (H-B-8, 1H) suggested the occurrence of other types of fatty acid moieties with isopropyl terminal branching structures. Furthermore, the molecular formula and index of hydrogen deficiency indicated that the fatty acid moiety is an 8-methylnonanoyl ester at the C-4 position of glucopyranose. To verify the structure, methyl 8-methylnonanoate (**7**) was synthesized (Scheme 1, Figure S16 in Supporting Information). Compound **5** was treated with

https://dx.doi.org/10.1021/acs.jnatprod.9b01234
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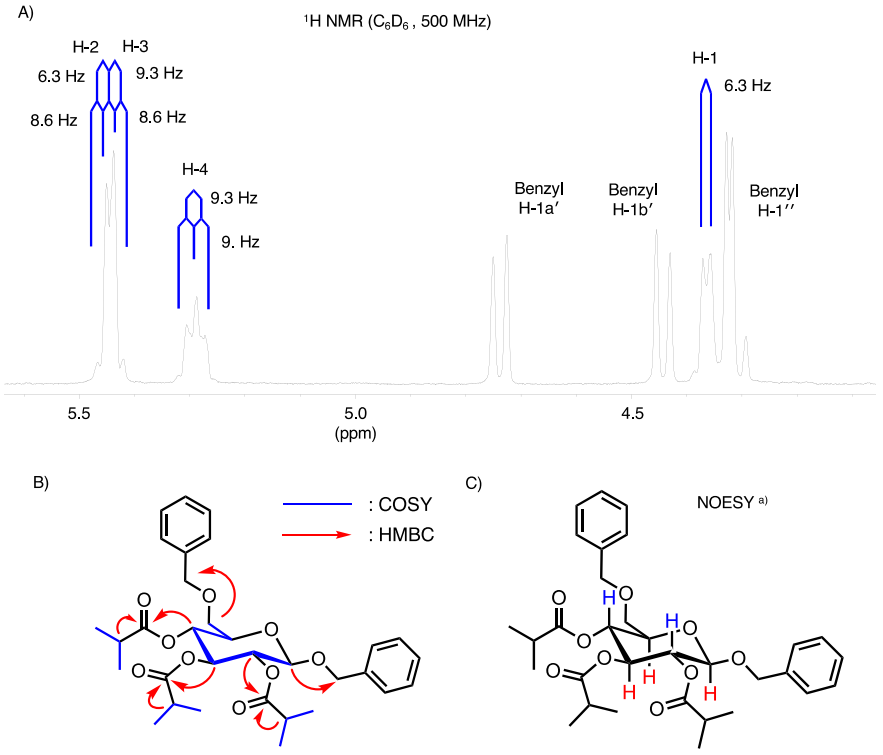

Figure 2. Key ^{1}H NMR resonances and COSY (blue), HMBC (red), and NOESY correlations of compound **4**. NOESY interactions were observed between each proton filled with the same color.

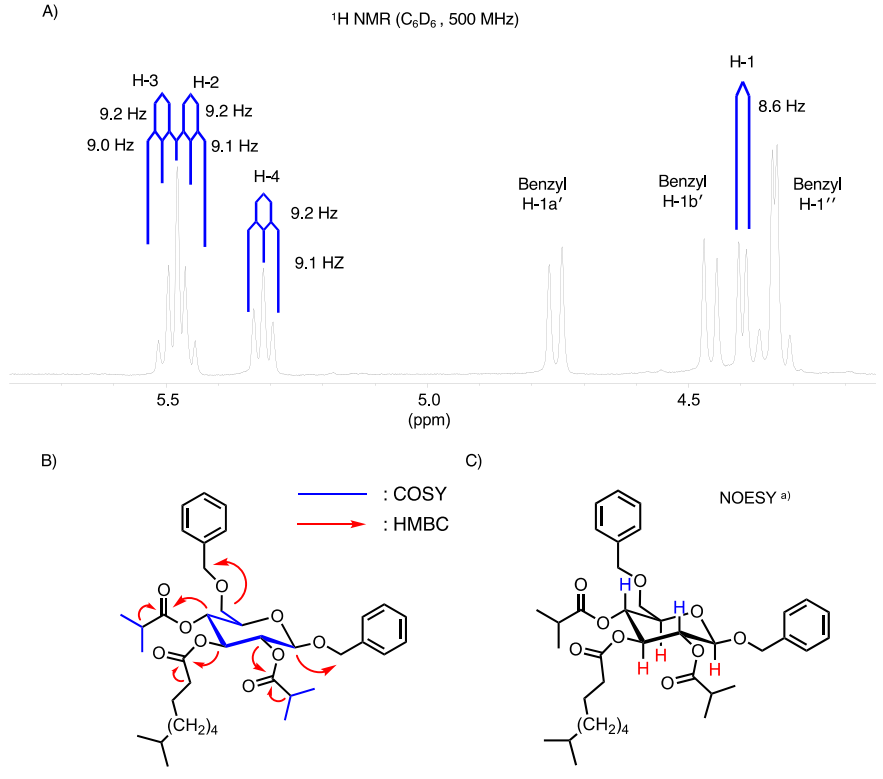

Figure 3. Key ^{1}H NMR resonances and COSY (blue), HMBC (red), and NOESY correlations of compound **5**. NOESY interactions were observed between each proton filled with the same color.

MeONa in anhydrous MeOH to give a crude mixture containing methyl esters, which was subjected to GC-MS analysis. The GC-MS chromatogram of the synthesized methyl 8-methylnonanoate (**7**) is given in Figure S17 (Supporting Information), which showed that the retention time of the target compound was at 12.2 min, and GC-MS features are given in Figure S18 (Supporting Information). The GC-MS chromatogram of the crude sample derived from compound **5**

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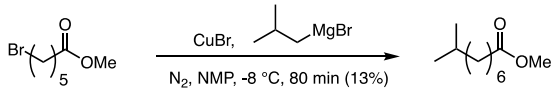
Scheme 1. Synthesis of Compound 7



Methyl 1-bromohexanoate — Methyl 8-methylnonanoate (**7**)

is given in Figures S19 and S20 (Supporting Information), in which a peak having almost the same GC-MS features as that of synthesized **7** was observed at 12.4 min. Thus, it was found that 8-methylnonanoate is attached to the basic glucopyranose structure at the C-3 position to form an ester. Finally, the HMBC correlations between the phenylmethylene protons of δ_H 4.75/4.46 (H-1a′ and -1b′) and anomeric carbon C-1 (δ_C 99.4) and between the phenylmethylene protons of δ_H 4.35/4.31 (H-1a″ and -1b″) and C-6 (δ_C 69.1) indicated that two benzyloxy groups are attached to the C-1 and C-6 positions. Therefore, it could be proposed that the chemical structure of **5** is 1,6-*O*-dibenzyl-3-*O*-(8-methylnonanoyl)-2,4-*O*-diisobutyryl-β-D-glucose. Analysis of the ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, and HMBC spectroscopic data gave the complete ^{1}H and ^{13}C NMR assignments for **5**, as listed in Table S2 (Supporting Information).

Compound **6** was isolated as a colorless oil. The molecular formula, $C_{38}H_{54}O_9$, was assigned from the HRFDMS data (*m*/*z* 654.3769 $[M]^+$, calcd 654.3767) (Figure S21, Supporting Information), based on which the index of hydrogen deficiency was once more calculated to be 12. The ^{1}H NMR resonances (Figure S22 and Table S3, Supporting Information) and the correlations observed in the COSY spectrum (Figure 4 and Figure S23, Supporting Information) between the signals at δ_H 4.39 (H-1, $J_{1,2}$ = 7.3 Hz), 5.47 (H-2, $J_{2,3}$ = 9.7 Hz), 5.49 (H-3, $J_{3,4}$ = 9.6 Hz), 5.32 (H-4, $J_{4,5}$ = 9.6 Hz), 3.43 (H-5), and 3.48 (H-6) together with their coupling constants suggested that the basic skeleton of **6** has a glucopyranose backbone, which was supported by HMBC correlations (Figures 4 and S24, Supporting Information). The NOESY correlations in Figures 4 and S25 showing H-1/H-3/H-5 cross-peaks substantiated this determination, and the δ_C 99.4 resonance in the ^{13}C NMR spectrum (Figure S26 and Table S3, Supporting Information) indicated a β anomeric structure. The correlations in the HMBC spectrum (Figures 4 and S24, Supporting Information) of H-2 (δ_H 5.47)/C-A-1(δ_C 174.5), H-3 (δ_H 5.49)/C-B-1 (δ_C 172.3), and H-4 (δ_H 5.32)/C-C-1(δ_C 174.6) were indicative of the linkage of the fatty acid moiety with the basic glucose structure. Furthermore, the correlations in the HMBC spectrum (Figures 4 and S24, Supporting Information) of H-A-2 (δ_H 2.45, methine proton)/C-A-1 (δ_C 174.5) and H-C-2 (δ_H 2.34, methine proton)/C-C-1(δ_C 174.6) together with the correlations in the COSY spectrum (Figures 4 and S23, Supporting Information) of δ_H 1.14−0.98 (H-A-3 and H-A-4) to 2.45 (H-A-2) and δ_H 1.14−0.98 (H-C-3 and H-C-4) to 2.34 (H−C-2) suggested that two isobutyryl esters are attached to the basic glucose structure. In addition, the molecular formula, the index of hydrogen deficiency, and the correlations in the HMBC spectrum (Figures 4 and S24, Supporting Information) of δ_H 2.22 (H-3, 2H)/δ_C 172.3 (C-B-1) together with the δ_H 0.91 (H-B-10, 3H, triplet) resonance in the ^{1}H NMR spectrum (Figure S22, Supporting Information) indicated the decanoyl ester moiety to be attached at the 3-position of the basic glucopyranose structure. Finally, the HMBC correlations between the phenylmethylene protons of δ_H 4.75/4.45 (H-

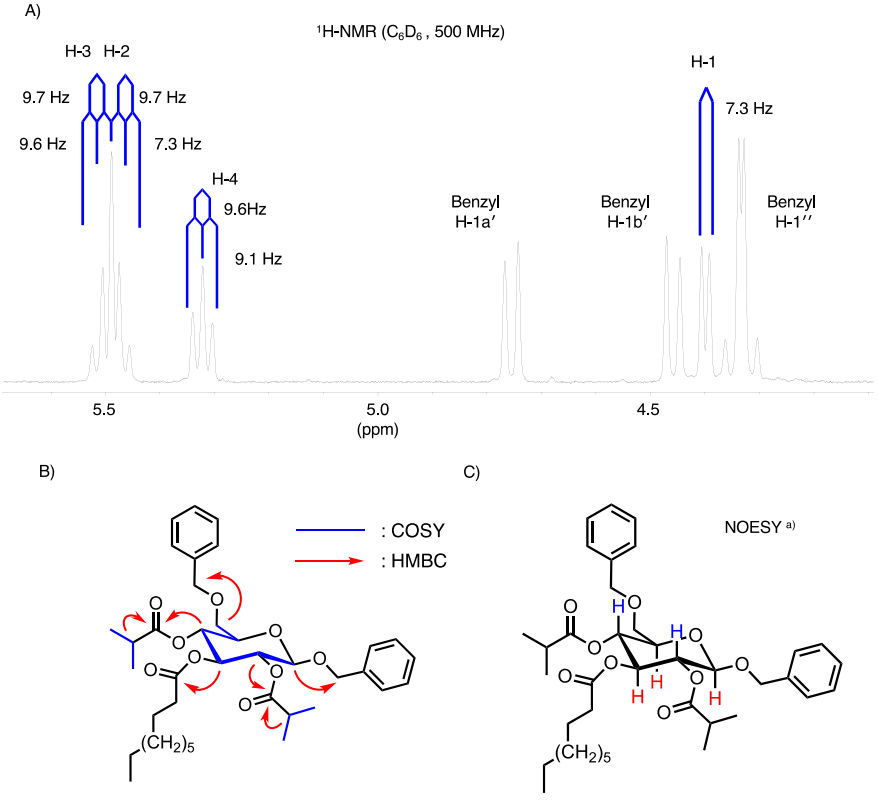


Figure 4. Key ^{1}H NMR resonances and COSY (blue), HMBC (red), and NOESY correlations of compound **6**. NOESY interactions were observed between each proton filled with the same color.

https://dx.doi.org/10.1021/acs.jnatprod.9b01234
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 1. NMR Spectroscopic Data for Compound 1 in $CDCl_3$ (500 MHz)

α anomer				β anomer			
position	δ_C	type	δ_H, mult. (*J* in Hz)	position	δ_C	type	δ_H, mult. (*J* in Hz)
1	90.2	CH	5.41 d (3.6)	1	95.7	CH	4.68, d (8.0)
2	71.2	CH	4.86, dd (10.9, 3.6)	2	73.3	CH	4.82, dd, (10.2, 8.0)
3	68.6	CH	5.59, dd (11.1, 10.9)	3	72.3	CH	5.32, dd (10.2 9.4)
4	68.5	CH	4.97, dd (11.1, 9.9)	4	68.3	CH	5.00, dd, (9.7, 9.4)
5	69.4	CH	4.00, br d (10.0)	5	74.4	CH	3.50, m
6	61.0	CH_2	3.70−3.60, m, 3.53−3.45, m	6	61.1	CH_2	3.70−3.60 m, 3.53−3.45, m
A-1	176.2	C		A1	177.2	C	
A-2	34.1−33.6	CH	2.51−2.38, m	A2	34.1−33.6	CH	2.51−2.38, m
A-3	19.4−18.5	CH_3	1.11−0.99, m	A3	19.4−18.5	CH_3	1.11−0.99, m
A-4	19.0−18.5	CH_3	1.11−1.01, m	A4	19.0−18.5	CH_3	1.11−1.01, m
B-1	175.8	C		B1	175.9	C	
B-2	34.1−33.6	CH	2.51−2.38, m	B2	34.1−33.6	CH	2.51−2.38, m
B-3	19.4−18.5	CH_3	1.11−0.99, m	B3	19.4−18.5	CH_3	1.11−0.99, m
B-4	19.0−18.5	CH_3	1.11−1.01, m	B4	19.0−18.5	CH_3	1.11−1.01, m
C-1	176.8	C		C1	176.4	C	
C-2	34.1−33.6	CH	2.51−2.38, m	C2	34.1−33.6	CH	2.51−2.38, m
C-3	19.4−18.5	CH_3	1.11−0.99, m	C3	19.4−18.5	CH_3	1.11−0.99, m
C-4	19.0−18.5	CH_3	1.11−1.01, m	C4	19.0−18.5	CH_3	1.11−1.01, m

Table 2. NMR Spectroscopic Data for Compound 2 in $CDCl_3$ (500 MHz)

α anomer				β anomer			
position	δ_C	type	δ_H, mult. (*J* in Hz)	position	δ_C	type	δ_H, mult. (*J* in Hz)
1	90.2	CH	5.42 d (3.2)	1	95.7	CH	4.68 d (7.7)
2	71.2	CH	4.80, dd (13.7, 3.2)	2	71.2	CH	4.78, dd (10.2, 7.7)
3	68.9	CH	5.61, dd (13.7, 10.3)	3	71.3	CH	5.32, dd (10.2 9.7)
4	68.8	CH	4.93, dd (10.3, 9.5)	4	73.5	CH	4.99, dd (9.7, 8.8)
5	70.4	CH	4.00, br d (9.0)	5	74.5	CH	3.48, m
6	61.1	CH_2	3.71−3.57, m, 3.54−3.45, m	6	70.5	CH_2	3.71−3.57, m, 3.54−3.45, m
A-1	177.2	C		A1	176.3	C	
A-2	34.1−33.6	CH	2.53, m	A2	34.1−33.6	CH	2.53, m
A-3	19.0−18.5	CH_3	1.11−1.01, m	A3	19.0−18.5	CH_3	1.11−1.01, m
A-4	19.0−18.5	CH_3	1.11−1.01, m	A4	19.0−18.5	CH_3	1.11−1.01, m
B-1	172.7	C		B1	172.7	C	
B-2	34.4−33.5	CH_2	2.15, 7 (7.0)	B2	34.4−33.5	CH_2	2.15, 7 (7.0)
B-3	29.1	CH_2	1.46, m	B3	29.1	CH_2	1.46, m
B-4	24.8	CH_2	1.23−1.10, m	B4	24.8	CH_2	1.23−1.10, m
B-5	29.4	CH_2	1.23−1.10, m	B5	29.4	CH_2	1.23−1.10, m
B-6	27.1	CH_2	1.23−1.10, m	B6	27.1	CH_2	1.23−1.10, m
B-7	38.9	CH_2	1.23−1.10, m	B7	38.9	CH_2	1.23−1.10, m
B-8	27.9	CH	1.43, m	B8	27.9	CH	1.43, m
B-9	22.4	CH_3	0.78, d (6.5)	B9	22.4	CH_3	0.78, d (6.5)
B-10	22.4	CH_3	0.78, d (6.5)	B10	22.4	CH_3	0.78, d (6.5)
C-1	176.8	C		C1	176.5	C	
C-2	34.1−33.6	CH	2.53, m	C2	34.1−33.6	CH	2.53, m
C-3	19.0−18.5	CH_3	1.11−1.01, m	C3	19.0−18.5	CH_3	1.11−1.01, m
C-4	19.0−18.5	CH_3	1.11−1.01, m	C4	19.0−18.5	CH_3	1.11−1.01, m

1a′ and -1b′) and anomeric carbon C-1 (δ_C 99.4) and between the phenylmethylene protons of δ_H 4.35/4.31 (H-1a″ and -1b″) and C-6 (δ_C 69.1) correspond with two substituted benzyloxy groups being attached at the C-1 and C-6 positions. Therefore, it was determined that the chemical structure of **6** is 1,6-*O*-dibenzyl-3-*O*-decanoyl-2,4-*O*-diisobutyryl-β-D-glucose. Analysis of the ^1H NMR, ^{13}C NMR, DEPT, HMQC, and HMBC spectroscopic data gave the complete ^1H and ^{13}C NMR assignments for **6**, as listed in Table S3 (Supporting Information).

Compounds **4** (25 mg), **5** (13 mg), and **6** (11 mg) were dissolved in ethyl acetate and mixed with palladium black under a hydrogen atmosphere with balloon pressure to give pennelliisides A (**1**, 7 mg), B (**2**, 11 mg), and C (**3**, 4 mg), respectively.

Pennelliiside A (**1**) was obtained as a colorless oil. The molecular formula, $C_{18}H_{30}O_9$, was determined from the HRFDMS data (*m*/*z* 391.1983 $[M]^+$, calcd 391.1968) (Figure S28, Supporting Information). Since the chemical structure of **4** was determined to be 1,6-*O*-dibenzyl-2,3,4-*O*-triisobutyryl-D-glucose, that of **1** should be 2,3,4-*O*-triisobutyryl-D-glucose.

https://dx.doi.org/10.1021/acs.jnatprod.9b01234
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 3. NMR Spectroscopic Data for Compound 3 in $CDCl_3$ (500 MHz)

α anomer				β anomer			
position	δ_C	type	δ_H, mult. (*J* in Hz)	position	δ_C	type	δ_H, mult. (*J* in Hz)
1	91.1	CH	5.41 d (3.6)	1	96.6	CH	4.68 d (8.0)
2	72.0	CH	4.83, dd (10.6, 3.6)	2	74.3	CH	4.83, br t (10.0)
3	69.8	CH	5.65, t (11.0)	3	72.1	CH	5.38, t (10.2)
4	69.5	CH	4.97, br. t (10.0)	4	69.3	CH	5.00, br t, (9.3)
5	70.4	CH	4.05, br d (10.6)	5	75.3	CH	3.54, m
6	61.9	CH_2	3.66, m, 3.55, m	6	62.0	CH_2	3.66, m, 3.55, m
A-1	177.1	C		A1	178.1	C	
A-2	34.6−34.9	CH	2.53, m	A2	34.6−34.9	CH	2.53, m
A-3	20.2−19.1	CH_3	1.15−1.08, m	A3	20.2−19.1	CH_3	1.15−1.08, m
A-4	20.2−19.1	CH_3	1.15−1.08, m	A4	20.2−19.1	CH_3	1.15−1.08, m
B-1	173.5	C		B1	173.5	C	
B-2	35.0	CH_2	2.20, dd (7.8, 7.5)	B2	35.0	CH_2	2.20, dd (7.8, 7.5)
B-3	25.6	CH_2	1.51, m	B3	25.6	CH_2	1.51, m
B-4 - B-6	29.7−30.7	CH_2	1.30−1.20, m	B4 - B-6	29.7−30.7	CH_2	1.30−1.20, m
B-7	29.7−30.7	CH_2	1.26, m	B7	29.7−30.7	CH_2	1.26, m
B-8	32.7	CH_2	1.28, m	B8	32.7	CH_2	1.28, m
B-9	23.5	CH_2	1.25, m	B9	23.5	CH_2	1.25, m
B-10	14.9	CH_3	0.85, t (7.3)	B10	14.9	CH_3	0.85, t (7.3)
C-1	177.7	C		C1	177.3	C	
C-2	34.6−34.9	CH	2.53, m	C2	34.6−34.9	CH	2.53, m
C-3	20.2−19.1	CH_3	1.15−1.08, m	C3	20.2−19.1	CH_3	1.15−1.08, m
C-4	20.2−19.1	CH_3	1.15−1.08, m	C4	20.2−19.1	CH_3	1.15−1.08, m

Burke et al. reported the isolation of 2,3,4-*O*-triisobutyryl-D-glucose from *S. pennellii*.[14] For convenience, compound **1** was named pennelliiside A, although the compound was previously reported. The reported ^1H and ^{13}C NMR spectra showed good accordance with those recorded in this study (Figures S29 and S30, Supporting Information). The COSY, HMQC, and HMBC spectra of **1** are given in Figures S31−S34, Supporting Information. Compound **1** contained α and β anomers, and it was revealed that the ratio of α and β anomers was ca. 2:1 from the peak area ratio of the resonances of the H-1 position in α and β anomers. The individual assignments of α and β anomers were clarified by the following procedure. The resonances of H-1 of α and β anomers were determined to be δ_H 5.41 and 4.68, respectively, due to the ^{13}C NMR chemical shifts of directly attached carbon with the protons, such as δ_C 90.2 for H-1α and δ_C 95.7 for H-1β together with their coupling constant values as 3.6 Hz of H-1α and 8.0 Hz of H-1β. Partial COSY spectra for **1** are given in Figure S32, which revealed the networks as H-1α (δ_H 5.41)/H-2α (δ_H 4.86)/H-3α (δ_H 5.59)/H-4α (δ_H 4.97)/H-5α (δ_H 4.00)/H-6α (δ_H 3.70−3.60 and 3.53−3.45) for the α anomer and H-1β (δ_H 4.68)/H-2β (δ_H 4.82)/H-3β (δ_H 5.32)/H-4β (δ_H 5.00)/H-5β (δ_H 3.50)/H-6β (δ_H 3.70−3.60 and 3.53−3.45) for the β anomer. Since there were overlapping resonances of acyl moieties, complete total assignments of the α and β anomers for each were not able to be accomplished. However, partial ^1H and ^{13}C NMR assignments for **1** are listed in Table 1.

Pennelliiside B (**2**) was obtained as a colorless oil. The molecular formula, $C_{24}H_{42}O_9$, was determined from the HRFDMS data (*m*/*z* 475.2889 $[M + H]^+$, calcd 475.2907) (Figure S35, Supporting Information). The ^1H NMR, ^{13}C NMR, COSY, HMBC, HSQC, and HSQC-TQCSY spectra (Figures S36−S42, Supporting Information) were complex features, but they also gave distinctive signals attributed to 2H multiplet methine protons at δ_H 2.53 (H-A-2 and H-C-2) and the 6H doublet terminal methyl protons at δ_H 0.78 (H-B-9/H-B-10) (Figure S35, Supporting Information). Since the chemical structure of **5** was determined to be 1,6-*O*-dibenzyl-3-*O*-(8-methylnonanoyl)-2,4-*O*-diisobutyryl-β-D-glucose, that of **2** should be 3-*O*-(8-methylnonanoyl)-2,4-*O*-diisobutyryl-D-glucose. Analysis of the ^1H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, and HSQC-TQCSY spectroscopic data provided only partial ^1H and ^{13}C NMR assignments for **2** due to the overlapping resonance of acyl moieties as listed in Table 2. However, assignments of α and β anomers attributed to the D-glucose structure were accomplished according to the procedure employed for compound **1**. Partial COSY spectra for **2** are given in Figure S39 (Supporting Information). The ratio of α and β anomers was ca. 2:1, which was deduced from the peak area ratio of the resonances of the H-1 position in the α and β anomers.

Pennelliiside C (**3**) was isolated as a colorless oil. The molecular formula, $C_{24}H_{42}O_9$, was determined from the HRFDMS data (*m*/*z* 475.2913 $[M + H]^+$, calcd 475.2907) (Figure S43, Supporting Information). Similar to pennelliiside A (**1**) and pennelliiside B (**2**), compound **3** showed complex resonances and cross-peaks in the ^1H NMR, ^{13}C NMR, COSY, HMBC, HSQC, and HSQC-TQCSYspectra (Figures S44−50, Supporting Information), but the signals were evident for 2H protons for each at δ_H 2.53 (m, H-A-2 and H-C-2) and the 3H triplet terminal methyl protons at δ_H 0.85 (H-B-10) (Figure S44, Supporting Information). Since the chemical structure of **6** was determined as 1,6-*O*-dibenzyl-3-*O*-decanoyl-2,4-*O*-diisobutyryl-β-D-glucose, that of **3** should be 3-*O*-decanoyl-2,4-*O*-diisobutyryl-D-glucose. Analysis of the ^1H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, and HSQC-TQCSY spectroscopic data yielded the partial ^1H and ^{13}C NMR assignments for **3** due to the overlapping resonance of acyl moieties as listed in Table 3. However, assignments of α and β anomers attributed to the D-glucose structure were accomplished according to the procedure employed for compound **1**. Partial COSY spectra for **3** are given in Figure S47 (Supporting

https://dx.doi.org/10.1021/acs.jnatprod.9b01234
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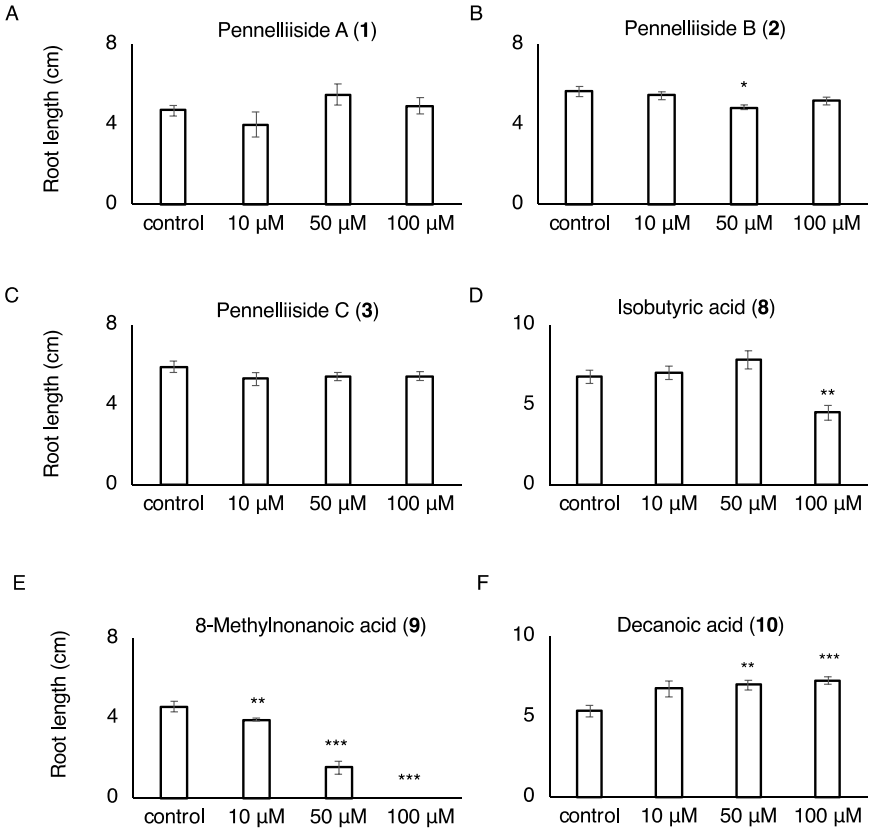

Figure 5. Root elongation assessment of acylglucose and their constituted fatty acids. Assays were performed by preparing MS medium (1/40 concentration) and mixing each test compound, (A) penelliside A (**1**), (B) penelliside B (**2**), (C) and penelliside C (**3**), and their constituent fatty acids, (D) isobutyric acid (**8**), (E) 8-methylnonanoic acid (**9**), and (F) decanoic acid (**10**). Error bars indicate ±SE (n = 8; results are from one representative experiment) (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ are significantly different by Welch's t-test).

Information). The ratio of α and β anomers was ca. 2:1, which was deduced from the peak area ratio of the resonances of the H-1 position in α and β anomers.

In a previous paper, Peterson et al. reported in 1997 that acyl sucrose acts as a phytoalexin to inhibit the seed germination of velvetleaf.[15] Thus, the biological activities of penelliside A (**1**), penelliside B (**2**), and penelliside C (**3**) were evaluated. Root elongation was assessed for **1**−**3** and their constituent fatty acids, isobutyric acid (**8**), 8-methylnonanoic acid (**9**), and decanoic acid (**10**). Compound **9** was synthesized according to a reported method,[16] except that methyl 7-bromoheptanoate was used, followed by treatment with a solution of NaOH in EtOH. The compounds tested were dissolved in MeOH to final concentrations of 10, 50, and 100 μM, and the seeds of *Arabidopsis thaliana* were used for the assay. The results are given in Figure 5; no activity was observed for penelliside A (**1**), penelliside B (**2**), penelliside C (**3**), and decanoic acid (**10**), although isobutyric acid (**8**) and 8-methylnonanoic acid (**9**) did show activity.

Since a fatty acid with a longer carbon chain with an isopropyl terminal branching structure showed more potent activity than that with a shorter carbon chain, an activity correlation test focusing on carbon chain length was performed. For the evaluation, fatty acids with isopropyl terminal branching structures, namely, isobutyric acid (**8**), isovaleric acid (**11**), 4-methylpentanoic acid (**12**), 5-methylhexanoic acid (**13**), 6-methylheptanoic acid (**14**), 7-methyloctanoic acid (**15**), and 8-methylnonanoic acid (**9**), were used. Compounds **14** and **15** were synthesized according to a reported method,[16] except that ethyl 5-bromopentanoate and methyl 6-bromohexanoate, respectively, were used, followed by treatment with a solution of NaOH in EtOH in each case, and the concentrations of the compounds were set to 50 μM. As a result, Figure 6, 8-methylnonanoic acid (**9**), 4-methylpentanoic acid (**12**), 5-methylhexanoic acid (**13**), 6-methylheptanoic acid (**14**), and 7-methyloctanoic acid (**15**) showed more potent activity than those of isobutyric acid (**8**) and isovaleric acid (**11**), even though isobutyric acid (**8**) showed a significant inhibitory effect on seed germination (Figure 5D). Figure 6

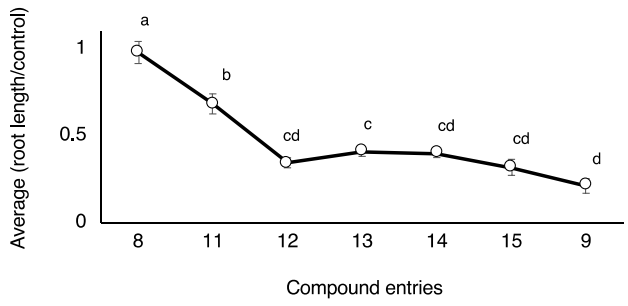

Figure 6. Activity correlation test between carbon chain length and root elongation inhibitory activity. *Arabidopsis thaliana* incubated for 2 weeks was used. MS medium (1/40 concentration) was prepared mixed with each saturated terminal branched fatty acids **8**, **9**, and **11**−**15**. Error bars indicate ±SE (n = 8; results are from one representative experiment). The plots that do not share the same letter differ significantly between the treatments ($p < 0.05$, Tukey−Kramer's test).

https://dx.doi.org/10.1021/acs.jnatprod.9b01234
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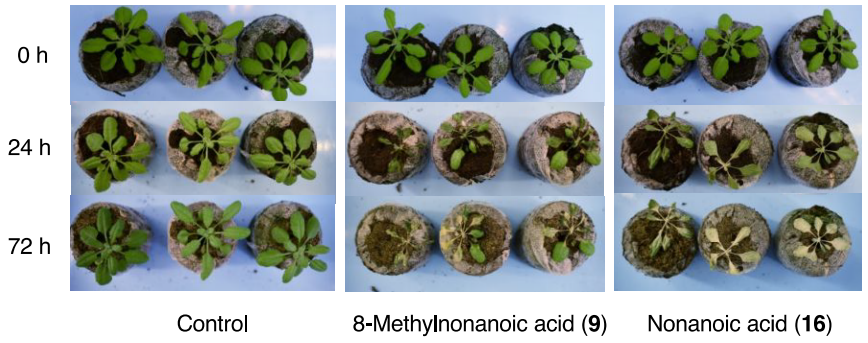

Figure 7. Herbicidal activity test of 8-methylnonanoic acid (**9**) and nonanoic acid (**16**). The spray solution was adjusted to a sample concentration of 5 mg/mL, ethanol 2%, Tween 20 0.3% and sprayed on *A. thaliana* grown for 30 days under long-day conditions.

indicates that the root length decreased as the length of the carbon chain increased up to six carbon atoms and remained almost the same for carbon chains of six and 10 carbon atoms, and 8-methylnonanoic acid (**9**) showed the most potent activity of the test compounds.

A weed-withering herbicide containing nonanoic acid (**16**) as a key ingredient is on the market. Therefore, it was assumed that 8-methylnonanoic acid (**9**) also has an ability to be used as an herbicide. An herbicidal activity test was performed on the basis of a previous report.[17] Solutions of the test samples (5 mg/mL), ethanol (2%), and Tween 20 (0.3%) were sprayed once on the leaves of *A. thaliana*. As a result, 8-methylnonanoic acid (**9**) showed approximately the same level of activity as nonanoic acid (**16**) (Figure 7).

In this investigation, one reported and two new acylglucoses named pennelliisides A (**1**), B (**2**), and C (**3**) were isolated from a wild tomato species, *S. pennellii*, although derivatization to a benzyl ether was needed to isolate the compounds in their pure forms. Since a reaction to remove the benzyl ether group using palladium black under a H_2 atmosphere was required to isolate the native compounds, the strategy adopted in this study cannot be used for isolation of compounds that react under the conditions of palladium black under a H_2 atmosphere. Although the adopted strategy had drawbacks, it was possible to isolate the compounds, discern the chemical nature of the compounds, and measure their physical and spectroscopic parameters. Based on the obtained information, synthesis of internal standards of the isolated compounds could be possible, which would enable endogenous amounts of the compounds to be evaluated in the observed biological events, such as whether the biosynthesis of acylglucose is affected by fluctuations of plant hormones or not. Furthermore, it was found that pennelliisides A (**1**), B (**2**), and C (**3**) did not have an inhibitory effect on root elongation itself, but isobutyric acid (**8**) and 8-methylnonanoic acid (**9**) exhibited such activity, which led to the hypothesis that their biological activity might be achieved when the plants withered. Nonanoic acid (**16**) is commercially used as an herbicide component, and it was very interesting to have discovered that *S. pennellii* uses a compound with a similar skeleton to combat other plants.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained with a JASCO P-2200 polarimeter. NMR spectra were recorded in $CDCl_3$ and benzene-d_6 using a JNM-EX 270 FT-NMR spectrometer (JEOL, ^{1}H NMR: 270 MHz, ^{13}C NMR: 67.5 MHz) and AMX 500 (Bruker, ^{1}H NMR: 500 MHz, ^{13}C NMR: 126 MHz). FDMS and FIMS analyses were performed on a JMS-T100GCV (JEOL) instrument. GC-MS analysis was completed on a Varian CP-3800 gas chromatograph with a Varian 1200L quadrupole MS/MS in electron ionization mode. Compounds **8**, **10**−**13**, and **16** and all reagents were purchased from Kanto Chemical Co., Inc.

Plant Material. Seeds of *Solanum pennellii* were obtained from the National Bioresource Project (NBRP, Tsukuba). The plants were grown in an artificial weather room (16 h light, 8 h dark, room temperature 25 °C) of the Faculty of Agriculture, Hokkaido University, Hokkaido, Japan, in 2018 and 2019. The plants were used for experiments approximately 80 days after sowing.

Extraction and Isolation. The aerial parts of the plants (1.7 kg) were rinsed by dipping the shoots in EtOH (5 L) for 30 s, and the epicuticular lipophilic extract was filtered and concentrated using a rotary evaporator, yielding a crude material. This crude material was then partitioned with EtOAc (500 mL) and saturated aqueous $NaHCO_3$ (500 mL). The organic layer was dried over $MgSO_4$ and concentrated, yielding a crude material, which was subjected to silica gel column chromatography (MeOH−$CHCl_3$−CH_3COOH, 5:95:0.1) to give a subfraction containing acylglucose (27 g). To a stirred mixture of the material containing acylglucose (4.5 g) in 1,4-dioxane (100 mL) were added TriBOT (300 mg) and TfOH (0.035 mL) under a N_2 atmosphere, and the reaction mixture was stirred for 16 h at room temperature. The volatile components of the reaction mixture were removed under reduced pressure to give an oil, which was subjected to silica gel column chromatography (100 g, EtOAc−*n*-hexane−CH_3COOH, 1:4:0.1) to give subfractions Fr-1 (1.0 g) and Fr-2 (4.1 g). Fr-1 and Fr-2 were subjected to HPLC (InertSustain C_{18}, 10 × 250 mm, 2.5 mL/min, $A_{210\ nm}$, MeOH−H_2O−CH_3COOH, 91:1:0.1) to give **4** (47 mg), **5** (33 mg), and **6** (17 mg).

*1,6-O-Dibenzyl-2,3,4-O-triisobutyryl-β-D-glucose (**4**).* Yellow oil; $[\alpha]^{20}_D$ +31 (*c* 0.5, $CHCl_3$); ^{1}H and ^{13}C NMR see Table S1, Figures S3 and S7 (Supporting Information); HRFDMS *m*/*z* 570.28225 $[M]^+$ (calcd for $C_{32}H_{42}O_9$ 570.28288).

*1,6-O-Dibenzyl-2,4-O-diisobutyryl-3-O-(8-methylnonanoyl)-β-D-glucose (**5**).* Oil; $[\alpha]^{20}_D$ −19 (*c* 0.6, $CHCl_3$); ^{1}H and ^{13}C NMR see Table S2, Figures S10 and S14 (Supporting Information); HRFDMS *m*/*z* 654.37501 $[M]^+$ (calcd for $C_{38}H_{54}O_9$ 654.37679).

*1,6-O-Dibenzyl-3-O-decanoyl-2,4-O-diisobutyryl-β-D-glucose (**6**).* Oil; $[\alpha]^{20}_D$ −16 (*c* 0.6, $CHCl_3$); ^{1}H and ^{13}C NMR see Table S3, Figures S22 and S26 (Supporting Information); HRFDMS *m*/*z* 654.37678 $[M]^+$ (calcd for $C_{38}H_{54}O_9$ 654.37679).

Synthesis of Methyl 8-Methylnonanoate (7). Compound **7** (560 mg, 3 mmol, 13%) was synthesized according to a reported method except for using methyl 7-bromoheptanoate.[16]

*Methyl 8-methylnonanoate (**7**).* ^{1}H NMR ($CDCl_3$, 270 MHz, Figure S16, Supporting Information) δ 3.53 (3H, s), 2.20 (2H, t, *J* = 7.1 Hz), 1.64−1.35 (3H, m), 1.30−1.00 (8H, m), 1.10−0.96 (2H, m), 0.76 (6H, d, *J* = 6.4 Hz); GC-MS found, *m*/*z* 186 (Figures S17 and S18, Supporting Information).

Analysis of Methyl 8-Methylnonanoyl Moiety in Compound 2. To a stirred solution of **5** (10 mg) in anhydrous MeOH (1 mL) was added a powder of NaOMe (10 mg), and the reaction mixture

https://dx.doi.org/10.1021/acs.jnatprod.9b01234
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was further stirred for 24 h. The usual workup was employed to give a solution containing methyl ester in hexane. A portion (1 μL) of the solution was analyzed using GC-MS in the following manner. For the analysis of the methyl ester using GC-MS, the injection temperature was 200 °C, and a fused-silica capillary column ($BetaDEX_{120}$; 30 m × 0.25 mm i.d., 0.25 μm film thickness; GL Sciences) was used. The temperature program started at 80 °C for 1 min and subsequently increased at 5 °C/min to 220 °C, which was maintained for 1 min. Helium was used as the carrier gas at a linear velocity of 1.2 mL/min, and all spectra were scanned within the range m/z 10−600. A GC-MS chromatogram and a chart of the MS pattern are given in Figures S19 and S20, Supporting Information, respectively.

Replacements of Benzyl Ether Groups to Give Compounds 1−3. To a stirred mixture of **4** (25 mg, 44 mmol) in EtOAc (1 mL) was added palladium black (4 mg) under a H_2 atmosphere with balloon pressure, and the reaction mixture was further stirred for 3 h at room temperature. The reaction mixture was filtered using Celite and concentrated under reduced pressure to give an oil, which was subjected to silica gel chromatography to give compound **1** (2,3,4-*O*-triisobutyryl-D-glucose, 7 mg, 19 mmol, 43%). Compounds **2** (2,4-*O*-diisobutyryl-3-*O*-(8-methylnonanoyl)-D-glucose, 11 mg, 23 mmol, 49%) and **3** (3-*O*-decanoyl-2,4-*O*-diisobutyryl-D-glucose, 4 mg, 8 mmol, 54%) were obtained following the same method, except that **5** (13 mg, 20 mmol) and **6** (11 mg, 17 mmol) were used, respectively.

Synthesis of Compounds 9, 14, and 15. Compounds **9**, **14**, and **15** were synthesized according to a reported method[16] except for using ethyl 6-bromohexanoate, ethyl 5-bromobutanoate, and ethyl 6-bromopentanoate followed by treatment with a solution of NaOH in EtOH to give **9** (78 mg, 4.5×10^{-1} mmol, 71%), **14** (1.1 g, 6.1 mmol, 54%), and **15** (2.0 g, 12 mmol, 59%), respectively.

*8-Methylnonanoic Acid (**9**).* ^{1}H NMR ($CDCl_3$, 270 MHz, Figure S51, Supporting Information) δ 2.34 (2H, t, J = 7.1 Hz), 1.74−1.42 (3H, m), 1.40−1.00 (8H, m), 0.86 (6H, d, J = 6.6 Hz).

*6-Methylheptanoic Acid (**14**).* ^{1}H NMR ($CDCl_3$, 270 MHz, Figure S52, Supporting Information) δ 2.28 (2H, t, J = 7.8 Hz), 1.67−1.37 (3H, m), 1.35−1.00 (4H, m), 0.80 (6H, d, J = 7.62 Hz).

*7-Methyloctanoic Acid (**15**).* ^{1}H NMR ($CDCl_3$, 270 MHz, Figure S53, Supporting Information) δ 2.23 (2H, t, J = 7.5 Hz), 1.60−1.31 (3H, m), 1.30−0.96 (6H, m), 0.76 (6H, d, J = 6.7 Hz).

Seed Growth Inhibition Bioassay. A root growth inhibition assay was performed in a 16 h/8 h photoperiod, and seedlings were scored on vertical plates with 1/40 MS medium. The seeds of *Arabidopsis thaliana* were used for the experiment. The seeds were cleaned to remove damaged, discolored, or foreign material. Each compound to be tested was dissolved in 4 μL of MeOH and added to the nutrient solution before autoclaving. The effects of each acylglucose or fatty acid treatment were assessed on 13-day-old seedlings.

Evaluation of Herbicidal Activity. *Arabidopsis thaliana* was used for the experiments. The plants were grown for 30 days at 23 °C. Herbicidal activity of the active compound and synthesized compounds was evaluated by foliar spraying. The carrier solution contained 2% ethanol and 0.3% Tween 20. A freshly prepared solution with the evaluated compound at a concentration of 5 mg/mL was used.[17] For spectroscopic studies, the spraying volume was dependent on the foliar surface area, ranging from approximately 1 to 3 mL/pot. Observations of withering were performed at 0, 24, and 72 h after the treatment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01234.

Experimental procedures, NMR spectra, MS data for compounds **1**−**7**, **9**, **14**, and **15** (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Mr. Y. Takata and Dr. E. Fukushi (Faculty of Agriculture, Hokkaido University) for assistance in obtaining the spectroscopic data.

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