New Toxic Metabolites from a Mushroom, *Hebeloma vinosophyllum*. I. Structures of Hebevinosides I, II, III, IV, and V

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The structures of hebevinosides I, II, III, IV, and V, new metabolites from a poisonous mushroom, *Hebeloma vinosophyllum*, were deduced to be $3\beta,16\beta$-dihydroxy-7β-methoxycucurbita-5,24-diene-3-O-β-D-xylopyranoside-16-O-(6-O-acetyl)-β-D-glucopyranoside (**1**), $3\beta,7\beta,16\beta$-trihydroxycucurbita-5,24-diene-3-O-(4-O-acetyl)-β-D-xylopyranoside-16-O-(6-O-acetyl)-β-D-glucopyranoside (**22**), $3\beta,7\beta,16\beta$-trihydroxycucurbita-5,24-diene-3-O-β-D-xylopyranoside-16-O-(6-O-acetyl)-β-D-glucopyranoside (**24**), $3\beta,16\beta$-dihydroxy-7β-methoxycucurbita-5,24-diene-3-O-β-D-xylopyranoside (**16**), and $3\beta,16\beta$-dihydroxy-7β-methoxycucurbita-5,24-diene-3-O-(4-O-acetyl)-β-D-xylopyranoside-16-O-(6-O-acetyl)-β-D-glucopyranoside (**27**), respectively, on the basis of chemical and spectral evidence, as well as the structure determination by X-ray crystallographic analysis of ethoxyhebevinogenin (**20**), obtained by enzymatic hydrolysis of **16**. Hebevinosides I, II, III, and V were proved to be toxic principles of this mushroom.

Keywords——Basidiomycetes; *Hebeloma vinosophyllum*; neurotoxin; mushroom toxin; hebevinoside; cucurbitane; triterpene glycoside

Several new triterpene glycosides named hebevinosides I, II, III, IV, and V (tentatively named HVA, H, I, D, and G in our previous report[1]) were isolated from aqueous methanolic extract of the fruit-bodies of a poisonous mushroom, *Hebeloma vinosophyllum* HONGO (Japanese name: Akahidawakafusatake). Hebevinosides I, II, III, and V were proved to be toxic principles of this mushroom, because of their lethal paralytic action in mice. We wish to report here the structures of hebevinosides I, II, III, IV, and V.

Hebevinoside I (**1**) [$C_{44}H_{72}O_{13}$, amorph., ultraviolet spectrum (UV): end absorption, infrared spectrum (IR) $_{max}^{KBr}$ cm^{-1}: 3400, 1735, 1635, 1075, 1040, positive to both the Liebermann–Burchard and Molisch reactions] gave a dihydro derivative (**2**), amorph., on catalytic hydrogenation, and acetylation of **2** afforded a dihydrohexaacetate (**3**), colorless needles from methanol, mp 176—177 °C, $[\alpha]_D^{22}$ +16 ° ($c=0.51$, MeOH), $C_{56}H_{86}O_{19}$ (the field desorption mass spectrum (FD-MS), M$^+$: m/z 1062, and elementary analysis).

Compound **1** afforded a per-O-methylated derivative (**4**) on methylation by Hakomori's method,[2] amorph., IR: no C=O, the ^{1}H nuclear magnetic resonance (^{1}H-NMR): 3.28 ppm (OMe), 3.40—3.61 ppm (OMe × 7). Compound **4** gave methyl 2,3,4-tri-O-methylxylopyranoside and methyl 2,3,4,6-tetra-O-methylglucopyranoside on methanolysis. The ^{1}H-NMR spectrum of **1** in pyridine-d_5 (C_5D_5N) indicated the presence of following partial structures: $\geqslant$C–CH$_3$ × 5 (δppm: 0.83, 1.14, 1.17, 1.24, 1.63, each 3H, s), >CH–CH$_3$ (1.06, 3H, d, $J=6.6$ Hz), –CH$_2$–CH=C(CH$_3$)$_2$ (1.70 and 1.74, each 3H, br s, 5.55, 1H, br t, $J=$

8.1 Hz), –CO–CH$_3$ (2.05, 3H, s), –O–CH$_3$ (3.41, 3H, s), $_{-O}^{-C}$>CH–CH=C (3.61 and 5.99, each

1H, d, $J=5.4$ Hz) and two anomeric H (4.69 and 4.79, each 1H, d, $J=8.1$ Hz). The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra of **1** in C_5D_5N and **3** in deuterochloroform

TABLE I. ^{13}C-NMR Data for **1** and **3**, δ (ppm) from TMS

		1 (in C_5D_5N)	**3** (in $CDCl_3$)
$-\underline{C}H_3$	$C-\underline{C}H_3$	16.3 17.9 18.5 19.8 25.9 25.9 28.9 29.2	15.5 18.4 19.4 22.6 22.8 25.4 28.1 28.6
	$CO-\underline{C}H_3$	20.8	20.6 (2C) 20.8 (5C)
	$O-\underline{C}H_3$	56.3	56.1
$-\underline{C}H_2-$	$C-\underline{C}H_2-C$	22.5 25.7 28.8 30.7 32.9 37.4 47.9	21.5 22.6 28.0 29.9 32.2 36.6 40.2 47.1
	$C-\underline{C}H_2-O$	64.7 66.8	61.5 62.0
$-\underline{C}H-$	$C-\underline{C}H-C$	30.7 39.8 49.0 55.5	28.1 29.5 38.9 47.7 53.1
	$C-\underline{C}H-O$	71.1 71.7 74.8 75.1 75.5 77.9 78.0 78.5 82.3 87.3	68.8 (2C) 70.7 70.8 71.5 71.7 73.1 77.2 82.1 86.6
	$O-\underline{C}H-O$	106.6 107.3	101.8 102.2 ($J=160$ Hz) ($J=163$ Hz)
	$C=\underline{C}H-C$	119.7 126.9	119.2
$-\underline{C}-$	$C-\underline{C}-C$	34.5 42.1 46.7 (2C)	33.9 41.3 46.0 46.2
	$C=\underline{C}-C$	130.1 148.0	146.8
	$C-\underline{C}O-O$	170.6	169.1 169.2 169.4 169.8 170.2 170.3 170.5

($CDCl_3$) showed the presence of various kinds of carbons as indicated in Table I. All of the above data suggested that **1** is a glycoside which consists of a tetracyclic triterpene and 1 mol each of xylose and glucose. The quantities of the methylated sugars obtained on methanolysis of **4** were too small for measurement of their optical activities. However, both sugars probably belong to the D-series because most sugars so far isolated from fungi are members of the D-series.[3]

On hydrolysis with 2 N hydrochloric acid in methanol, **1** afforded an aglycone (**5**), colorless needles, mp 169—171 °C, $C_{31}H_{52}O_3$ (electron impact high-resolution mass spectrum (EI-high MS) m/z, M^+: obsd., 472.392, calcd., 472.391), UV λ_{max}^{MeOH} nm (log ε): 235 (4.09), 242 (4.13), 251 (3.96) (a heteroannular diene system), IR_{max}^{KBr}: 3400 cm^{-1} (OH). Signals of two olefinic methyls, a methoxyl, and a $\begin{smallmatrix}-C\\-O\end{smallmatrix}>CH-CH=C$ group as well as a xylosyl, a glucosyl, and an acetyl group, which were observed in the spectrum of **1**, disappeared and signals of two tertiary methyls, a different methoxyl, and two olefinic hydrogens newly appeared in the spectrum of **5**. It was suggested that a methoxyl group derived from methanol was newly added to the olefinic carbon bearing two methyls during the acid hydrolysis. Spin decoupling measurement of the ^{1}H-NMR spectrum of **5** indicated that a carbon bearing a secondary hydroxyl group, a methylene, a conjugated diene containing two olefinic hydrogens, and another methylene may be present in the partial structure A) in this compound, as shown in Chart 1. The evidence suggested that the cucurbitane-type skeleton should be selected as a possible carbon skeleton of **5** among several known tetracyclic triterpene skeletons, because the presence of the partial structure A) is compatible with a cucurbitane structure. In this case H_a (3.43 ppm) is assignable to position 3, H_b and H_c (2.37 and 2.55 ppm) to 2, H_d (5.47 ppm) to 1, H_e (5.58 ppm) to 6, and H_f and H_g (2.07 and 2.15 ppm) to 7 in **5** as shown in Chart 1. The coupling pattern of H_a (dd, $J_1=9$, $J_2=6$ Hz) suggested that the hydroxyl group at position 3 has β-configuration. The ^{1}H-NMR spectrum of **5** showed that a signal of another hydrogen on the carbon bearing a secondary hydroxyl group is present at 4.37 ppm (dd, $J_1=13$, $J_2=6$ Hz).
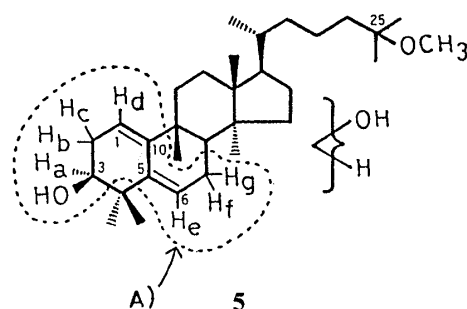
^{1}H-NMR, δ (ppm)

H_a: 3.43 (dd, $J_1 = 9$, $J_2 = 6$ Hz)

H_b, H_c: 2.37 (m), 2.55 (m)

H_d: 5.47 (br s)

H_e: 5.58 (br s)

H_f, H_g: 2.07, 2.15 (each 1H, m)

Chart 1

On acetylation, **5** afforded a monoacetate (**6**) and a diacetate (**7**). The ^{1}H-NMR spectrum of **6** showed that the signal of an acetyl methyl appeared at 2.05 ppm (s) and that of the hydrogen on the carbon bearing a hydroxyl group at position 3 (see Chart 2) was shifted to 4.67 ppm (1H, dd, $J_1 = 9.5$, $J_2 = 6.1$ Hz). The ^{1}H-NMR spectrum of **7** indicated that the signals of two hydrogens on the carbons bearing hydroxyl groups at position 3 and at another unknown position (see Chart 2) were shifted to 4.67 (1H, dd, $J_1 = 9.1$, $J_2 = 6.1$ Hz) and 5.28 ppm (1H, dd-like broad peak, $W_{1/2} = 10.8$ Hz). These spectral data showed that **6** is a monoacetylated derivative of the hydroxyl group at position 3 of **5**, and **7** is a diacetylated derivative of both hydroxyl groups. Oxidation of **6** with chromium trioxide in pyridine afforded a monoacetyl ketone (**8**), ^{13}C-NMR δ (ppm) in CDCl$_3$: 21.0 (acetyl $\underline{C}$H$_3$), 170.8 (acetyl $\underline{C}$=O), 220.2 (ketone $\underline{C}$=O). Irradiation of a signal at 1.61 ppm (1H, m) in spin decoupling measurement of the ^{1}H-NMR spectrum of **8** caused transformations of both doublets of a secondary methyl at 0.87 ppm (a secondary methyl is present at position 20 in the cucurbitane-type compound **8**) and a hydrogen at 2.14 ppm (1H, d, $J = 8.6$ Hz) to singlets. The hydrogen at 2.14 ppm is assigned as H-17 neighboring the carbonyl group in **8**. Accordingly, the carbonyl group is present at position 16 in **8** as shown in Chart 2. The original secondary hydroxyl group was concluded to be present at position 16 in **6** (and also in **5**). The coupling pattern of H-16 (4.37 ppm, dd, $J_1 = 13$, $J_2 = 6$ Hz) of **5** suggested that the hydroxyl group has β-configuration.

Thus, the structures of **5**, **6**, and **7** were concluded to be as illustrated in Chart 2. On similar hydrolysis with 2 N hydrochloric acid in methanol, the dihydro derivative (**2**) of **1** afforded an aglycone (**9**) [mp 117—120 °C, C$_{30}$H$_{50}$O$_2$ (electron impact mass spectrum (EI-MS) m/z (%): 442 (100, M$^+$)), UV λ_{max}^{MeOH} nm (log ε): 235 (4.10), 241.5 (4.15), 251 (3.94), IR$_{max}^{KBr}$: 3400 cm^{-1} (OH)], which also gave a monoacetate (**10**) and a diacetate (**11**) on acetylation. The ^{1}H-NMR spectra of **9**, **10**, and **11** and also the ^{13}C-NMR spectrum of **9** indicated that they have no methoxyl group at position 25, although **5**, **6**, and **7** have methoxyls.
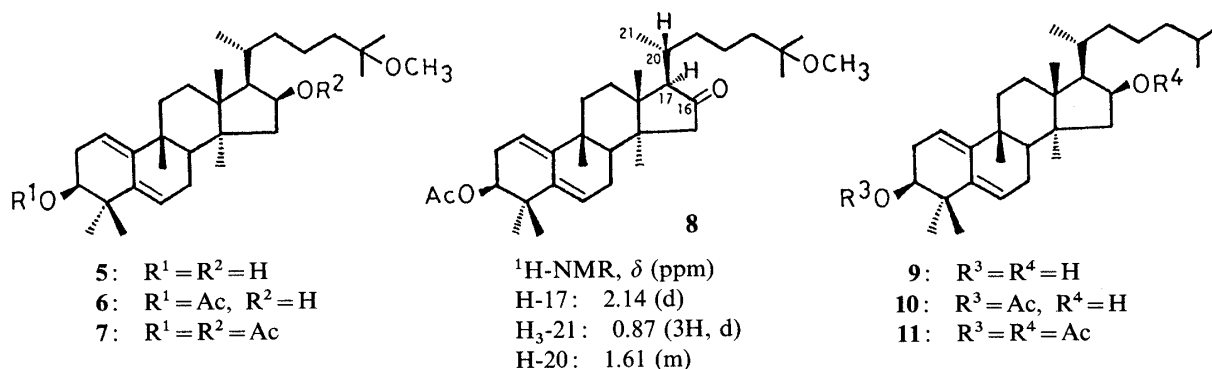

5:   R^1 = R^2 = H
6:   R^1 = Ac, R^2 = H
7:   R^1 = R^2 = Ac

^{1}H-NMR, δ (ppm)
H-17:   2.14 (d)
H$_3$-21:   0.87 (3H, d)
H-20:   1.61 (m)

9:   R^3 = R^4 = H
10:   R^3 = Ac, R^4 = H
11:   R^3 = R^4 = Ac

Chart 2

On treatment with 0.1% potassium hydroxide in methanol, **1** afforded a desacetyl derivative (**12**), IR: no carbonyl. The ^{13}C-NMR spectrum of **12** indicated that the signals of C-4, C-5, C-6 in the glucosyl moiety had shifted to 72.0 (+0.3), 78.2 (+2.7), and 63.2 (−1.5) ppm from 71.7, 75.5, and 64.7 ppm of the corresponding carbons in **1** as a result of the loss of the acetyl group (see Table II). These results are compatible with the assumption that the acetyl group is attached to C-6 of glucose in **1**, according to the acetylation shift rule.[4] Measurement of the EI-MS of the pertrimethylsilylated derivative of **1** afforded predominant fragment ions, m/z 421 ($C_{17}H_{37}O_6Si_3{}^+$) and 349 ($C_{14}H_{33}O_4Si_3{}^+$), which might be formed by fission of the pertrimethylsilylated 6-acetylglucosyl and xylosyl moieties of **1**.[5] Thus, the methoxyl group of **1** was supposed to be present in the aglycone part, but not in the sugar moieties. The elimination of a methoxyl group as well as the sugar moieties from **1** afforded **5**, which contains a cucurbita-1 (10), 5-diene system, indicating that the presence of the 7-methoxycucurbit-5-ene system in **1** is reasonable. The coupling pattern of H-7 (3.61 ppm, d, $J = 5.4$ Hz) in **1** in the ^{1}H-NMR spectrum suggested that the methoxyl group at position 7 has β-configuration.

On enzymatic hydrolysis with Molsin[6] in 25% ethanolic buffer solution (pH 4.0), **12** afforded a partially hydrolyzed product (**13**). Comparison of the ^{1}H- and ^{13}C-NMR spectra of **13** with those of **12** indicated that replacement of the methoxyl group by ethoxyl occurred at position 7 in addition to the removal of xylose (glucose remained) but no further change occurred during this reaction. The signals of H-6 and -7 in the ^{1}H-NMR spectrum of **13** were not very different from those of **12** and agreed well with those of **1**. Further, the signals of C-6 and -7 in the ^{13}C-NMR spectrum of **13** were very similar to those of **12** and **1** (see Experimental). The β-configuration of the 7-ethoxyl group in **13** was indicated from the chemical shifts and coupling constants of H-6 (5.98 ppm, d, $J = 5.0$ Hz) and H-7 (3.62 ppm, d, $J = 5.0$ Hz), which agreed well with those of **1** having a 7β-methoxyl group at position 7.

It is known that the signals of alkyl carbons bearing a hydroxyl group are generally observed to shift to lower magnetic field in ^{13}C-NMR spectra when they are etherized. An example of C-3 bearing a hydroxyl group in the cucurbitane-type triterpene has been reported, in which the signal appeared at 75.3 ppm but shifted to 85.8 ppm upon glycosidation of the hydroxyl group.[7] In the cucurbitane-type triterpenes, the signal of C-16 bearing a hydroxyl group (e.g. in compound **14**) was observed at 71.5 ppm[8] but that of compound **15**, which is etherized, was observed at 82.0 ppm[9] as illustrated in Chart 3.
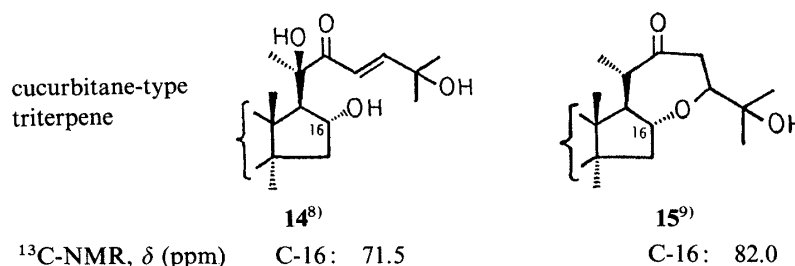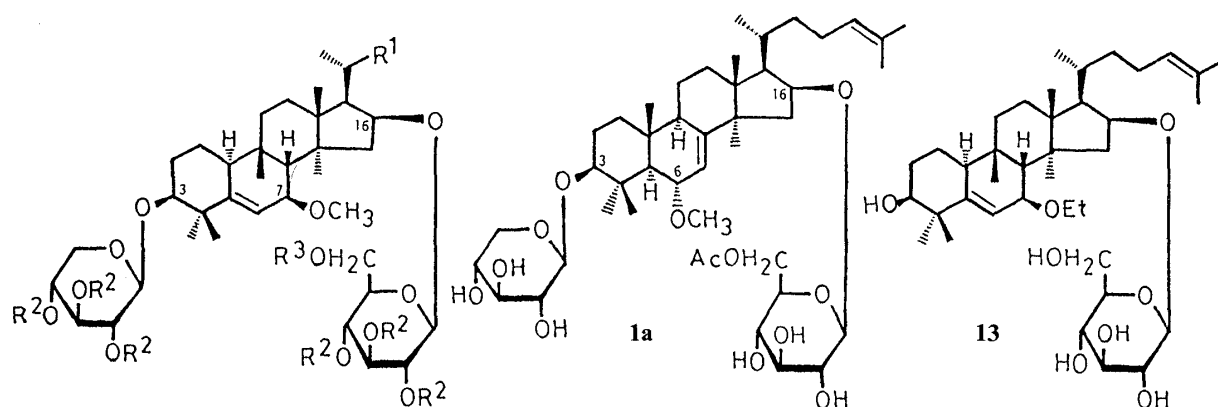
cucurbitane-type
triterpene

^{13}C-NMR, δ (ppm)   C-16: 71.5   C-16: 82.0

14[8]   **15**[9]

Chart 3

The glucosylated carbon in **13** is observed at 82.1 ppm (not around 85.8 ppm) in the ^{13}C-NMR spectrum, indicating that glucose is attached to C-16, not C-3. Accordingly, the structure of hebevinoside I is deduced to be **1**, in which xylose and 6-O-acetylglucose are attached to C-3 and C-16 of the cucurbitane-type skeleton, respectively, as shown in Chart 4. The coupling constants of the two anomeric hydrogens of **1** (each $J = 8.1$ Hz) in the ^{1}H-NMR spectrum and of the two anomeric carbons of **3** (160 and 163 Hz) in the ^{13}C-NMR spectrum (see Table I) indicated that xylose and 6-O-acetylglucose are attached to C-3 and C-16, respectively, both in the mode of β-configuration.[10]

Chart 4

1: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = H$, $R^3 = Ac$
2: $R^1 = CH_2CH_2CH_2CH(CH_3)_2$, $R^2 = H$, $R^3 = Ac$
3: $R^1 = CH_2CH_2CH_2CH(CH_3)_2$, $R^2 = R^3 = Ac$
4: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = R^3 = Me$
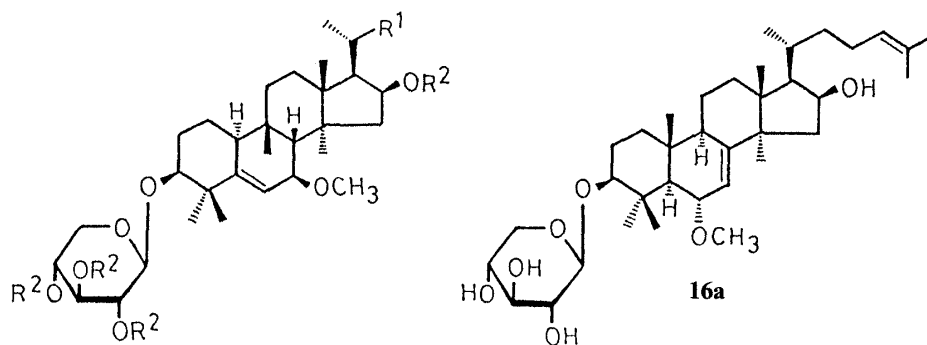12: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = R^3 = H$

Many cucurbitane-type compounds have so far been isolated exclusively from higher plants, especially from several plants belonging to Cucurbitaceae[11a] Cruciferae,[11a] and Primulaceae.[11b] On the other hand, many lanostane-type compounds have been isolated from fungi, especially from those belonging to Basidiomycetes.[12] Furthermore, it is well known that certain lanostane-type compounds are transformed into cucurbitane-type compounds on treatment with a strong Lewis acid such as boron trifluoride because of migration of the methyl group at position 10 to the carbonium ion formed at position 9.[13] Therefore, an alternative structure consisting of a lanostane-type skeleton (1a) should also be considered for hebevinoside I. The spectral data of 1a might be quite similar to those of 1.

Hebevinoside IV (16) [colorless needles from methanol, mp 172—173 °C, $C_{36}H_{60}O_7$ (FD-MS m/z: 604 (M$^+$)), UV: end absorption, IR$_{max}^{KBr}$cm^{-1}: 3410, 1638, 1075, 1038] was positive to both the Liebermann–Burchard and Molisch reactions. On catalytic hydrogenation, 16 afforded a dihydro derivative (17), and on acetylation, a tetraacetate (18). Catalytic hydrogenation of 18 gave a dihydrotetraacetate (19), mp 122—125 °C, $[\alpha]_D^{17}$ +64° (c=0.081, MeOH).

Comparison of the ^{1}H- and ^{13}C-NMR spectra of 16 with those of 1 indicated that the signals of the 6-O-acetylglucosyl moiety disappeared and the signal of C-16 of 16 was shifted 11.2 ppm to higher magnetic field (71.1 ppm) from that (82.3 ppm) in 1 (see Table II). This suggested that 16 lacks the 6-O-acetylglucose at position 16 as compared with 1. Therefore, the structure 3β,16β-dihydroxy-7β-methoxycucurbita-5,24-diene-3-O-β-D-xylopyranoside (16) was supposed for hebevinoside IV, as shown in Chart 5. However, the possibility of the lanostane-type structure 16a still remained for hebevinoside IV (see Chart 5). Thus, we decided to obtain the aglycone of hebevinoside IV by enzymatic hydrolysis with Molsin[6] in the same way as used to get 13 from 12, in order to provide a crystalline sample for X-ray analysis.

On hydrolysis with Molsin[6] in 50% ethanolic buffer solution (pH 4.0), 16 afforded an aglycone (20), colorless prisms from C_6H_6, mp 174—175 °C, $[\alpha]_D^{25}$ +21° (c=0.11, CHCl$_3$), $C_{32}H_{54}O_3$ (EI-high MS, m/z, M$^+$: obsd., 486.404, calcd., 486.407), UV: end absorption. Comparison of the ^{1}H- and ^{13}C-NMR spectra of 20 with those of 16 (see Experimental) suggested that the replacement of the methoxyl group by ethoxyl occurred as well as hydrolytic elimination of xylose from 16, but no further change was observed in terms of the spectral data. Formation of 20 from 16 seems to occur through a route (without any transformation of

16: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = H$
17: $R^1 = CH_2CH_2CH_2CH(CH_3)_2$, $R^2 = H$
18: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = Ac$
19: $R^1 = CH_2CH_2CH_2CH(CH_3)_2$, $R^2 = Ac$

Chart 5

TABLE II. ^{13}C-NMR Data for Hebevinosides, δ (ppm) from TMS in C_5D_5N

Position	Hebevinoside				
	I (1)	II (22)	III (24)	IV (16)	V (27)
3	87.3	87.4	87.4	87.4	87.4
5	148.0	146.2	146.3	147.9	147.8
6	119.7 ·	122.5	122.4	119.3	119.5
7	77.9	67.5	67.5	77.8	77.8
16	82.3	82.2	82.2	71.1	82.2
24	126.9	126.8	126.8	126.2	126.9
25	130.1	130.0	129.9	130.5	130.0
OMe	56.3			56.2	56.3
Xylose					
-1	107.3	107.3	107.7	107.8	107.5
-2	74.8	74.9	75.1	74.9	75.0
-3	78.5	74.9	78.4	78.5	74.8
-4	71.1	73.1	71.1	71.2	73.1
-5	66.8	63.1	66.8	67.1	63.2
Glucose					
-1	106.6	106.5	106.6		106.8
-2	75.1	75.2	75.0		75.0
-3	78.0	78.3	78.6		78.3
-4	71.7	71.7	71.9		71.7
-5	75.5	75.4	75.6		75.4
-6	64.7	64.7	64.8		64.5
CO$\underline{C}$H$_3$	20.8	20.8	20.8		20.9
		20.8			20.9
$\underline{C}$OCH$_3$	170.6	170.5	170.8		170.5
		170.7			170.6

the skeleton) similar to that in the formation of **13** from **12**. Comparison of the ^1H-NMR data of **20** (H-7: 3.46 ppm, br d, $J = 5.2$ Hz, and H-6: 5.72 ppm, d, $J = 5.2$ Hz) with those of **16** itself (H-7: 3.42 ppm, br d, $J = 5.6$ Hz, and H-6: 5.74 ppm, d, $J = 5.6$ Hz) indicated that the ethoxyl group at C-7 in **20** has the same configuration as the 7-methoxyl in **16**. Accordingly, the structure of the aglycone (**20**) was deduced to be $3\beta,16\beta$-dihydroxy-7β-ethoxycucurbita-5,24-diene. However, if hebevinoside IV has the structure **16a**, the aglycone should be $3\beta,16\beta$-dihydroxy-6α-ethoxylanosta-7,24-diene (**20a**) (see Chart 6). Thus, if this aglycone has the

structure **20**, the structure of the common aglycone of hebevinosides I and IV may be deduced to be 3β,16β-dihydroxy-7β-methoxycucurbita-5,24-diene (**21**), and if it has the structure **20a**, the common aglycone of hebevinosides I and IV may be deduced to be 3β,16β-dihydroxy-6α-methoxylanosta-7,24-diene (**21a**) (see Chart 6).

For X-ray crystallographic analysis of **20** [orthorhombic, space group $P2_12_12$, lattice constants $a = 12.769$ (4), $b = 20.395$ (9), $c = 12.170$ (4) Å, $Z = 4$, $D_{obsd.} = 1.05$ g/cm^3 for $D_{calcd.} = 1.02$ g/cm^3], the data on 1789 independent reflections ($|F_o| > 2\sigma(|F_o|)$) within the range of $2° \leqq 2\theta \leqq 130°$, measured using Cu$K\alpha$ radiation, were solved directly by the MULTAN program,[14] and the solution was refined by the block-diagonal least-squares method with isotropic and anisotropic temperature factors for all non-hydrogen atoms to give a final R-value of 0.142, excluding the contributions of all hydrogen atoms in **20**. The final fractional co-ordinates of all non-hydrogen atoms with estimated standard deviations are listed in Table III. The solved structure of the aglycone from **16** showed that its relative stereostructure is not identical with the relative stereostructure of **20a** but with that of **20** (3β,16β-dihydroxy-7β-ethoxycucurbita-5,24-diene), as shown in Fig. 1 and Chart 6. Accordingly, the structure of the aglycone of **16** and of **1** is deduced to be 3β,16β-dihydroxy-7β-methoxycucurbita-5,24-diene (**21**), which we wish to call methoxyhebevinogenin, and that of **20**, ethoxyhebevinogenin. Thus, the structures of hebevinosides I and IV are deduced to be **1** and **16**, both of which have **21** as the common aglycone. Thus, the above X-ray crystallographic analysis was adequate for our purpose, though the final R-value of this analysis was not low enough.

Hebevinoside II (**22**) [amorph., $[\alpha]_D^{20}$ $+30°$ ($c = 0.20$, acetone), UV: end absorption, IR$_{max}^{KBr}$cm^{-1}: 3400, 1735, 1640, 1075, 1035] afforded a hexaacetate (**23**) on acetylation.

Hebevinoside III (**24**) [amorph., UV: end absorption, IR$_{max}^{KBr}$cm^{-1}: 3400, 1735, 1640, 1080, 1040] afforded a heptaacetate on acetylation. Catalytic hydrogenation of the heptaacetate afforded a dihydroheptaacetate (**25**), mp 204—205 °C, $[\alpha]_D^{20}$ $+89°$ ($c = 0.058$, MeOH). The heptaacetate of **24** was identical with the hexaacetate (**23**) derived from **22** in terms of the ^{1}H-NMR and IR spectra and thin layer chromatographic (TLC) behavior. Furthermore, the permethylether of **24** obtained by Hakomori's method[2] was identical with the permethylether (**4**) derived from **1** in terms of IR spectra and TLC behavior. In the ^{1}H-NMR spec-

TABLE III.   Fractional Coordinates of Non-hydrogen Atoms with Estimated
Standard Deviations in Parentheses

Atom	x	y	z	Atom	x	y	z
C-1	0.398 (1)	0.357 (1)	0.706 (1)	C-19	0.418 (1)	0.227 (1)	0.607 (1)
C-2	0.374 (1)	0.429 (1)	0.719 (1)	C-20	0.011 (1)	0.093 (1)	0.904 (1)
C-3	0.331 (1)	0.460 (1)	0.617 (1)	C-21	0.071 (1)	0.109 (1)	1.009 (1)
C-4	0.237 (1)	0.424 (1)	0.574 (1)	C-22	−0.108 (1)	0.089 (1)	0.932 (1)
C-5	0.252 (1)	0.347 (1)	0.572 (1)	C-23	−0.154 (1)	0.157 (1)	0.961 (2)
C-6	0.220 (1)	0.312 (1)	0.482 (1)	C-24	−0.270 (2)	0.151 (1)	0.998 (2)
C-7	0.209 (1)	0.239 (1)	0.484 (1)	C-25	−0.359 (2)	0.169 (1)	0.954 (2)
C-8	0.227 (1)	0.206 (1)	0.597 (1)	C-26	−0.364 (3)	0.195 (1)	0.833 (2)
C-9	0.315 (1)	0.242 (1)	0.662 (1)	C-27	−0.458 (1)	0.167 (1)	1.003 (2)
C-10	0.296 (1)	0.316 (1)	0.672 (1)	C-28	0.144 (1)	0.438 (1)	0.653 (2)
C-11	0.323 (1)	0.212 (1)	0.785 (1)	C-29	0.205 (2)	0.452 (1)	0.457 (1)
C-12	0.216 (1)	0.199 (1)	0.841 (1)	C-30	0.067 (1)	0.261 (1)	0.679 (1)
C-13	0.149 (1)	0.155 (1)	0.769 (1)	C-31	0.243 (2)	0.154 (1)	0.354 (1)
C-14	0.125 (1)	0.196 (1)	0.661 (1)	C-32	0.307 (2)	0.136 (1)	0.258 (2)
C-15	0.047 (1)	0.148 (1)	0.600 (1)	O-3	0.415 (1)	0.458 (0)	0.536 (1)
C-16	−0.027 (1)	0.127 (1)	0.700 (1)	O-7	0.277 (1)	0.214 (0)	0.401 (1)
C-17	0.031 (1)	0.143 (1)	0.809 (1)	O-16	−0.057 (1)	0.058 (0)	0.690 (1)
C-18	0.202 (1)	0.090 (1)	0.750 (1)				

Fig. 1.   Relative Stereostructure of **20**

20:	$R^1 = Et$
21:	$R^1 = Me$
26:	$R^1 = H$

| **20a**: | $R^2 = Et$ |
| **21a**: | $R^2 = Me$ |
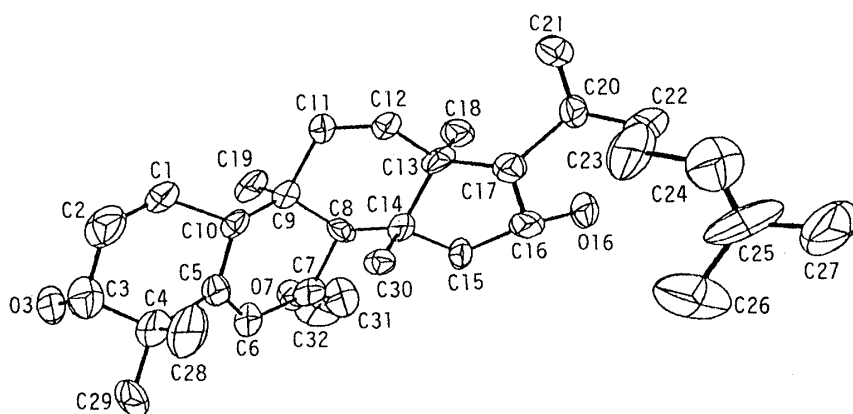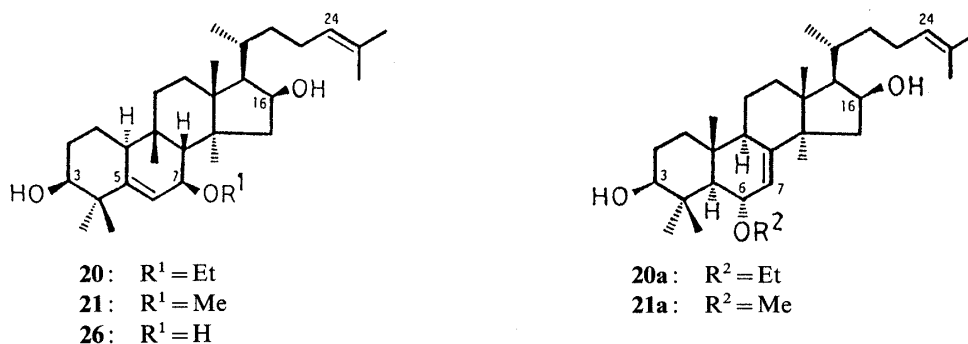
Chart 6

trum of **24**, no methoxyl group signal appeared and the signal of a methine hydrogen bearing a hydroxyl group was observed at 4.41 ppm (d, $J = 5.6$ Hz), coupled with an olefinic hydrogen at position 6 (6.12 ppm, d, $J = 5.6$ Hz). A comparison of the ^{13}C-NMR spectra of **24** and **1** showed that the methoxyl signal observed at 56.3 ppm in **1** had disappeared and that of the carbon bearing a hydroxyl group at position 7 was shifted to 67.5 (-10.4) ppm in **24**, supporting the above findings. Therefore, hebevinoside III (**24**) was deduced to be the desmethyl derivative of **1**, as shown in Chart 7.

The above data suggested that **22** is a monoacetyl derivative of **24**. It was indicated that the 4-hydroxyl group in the xylosyl moiety of **22** is acetylated in addition to the 6-hydroxyl group in the glucosyl moiety since the signal of C-4 of xylose was shifted to 73.1 ($+2.0$) ppm and those of C-3 and C-5 to 74.9 (-3.5) and 63.1 (-3.7) ppm in the ^{13}C-NMR spectrum of **22** (see Table II). Accordingly, the structure of hebevinoside II was deduced to be **22** as shown in Chart 7. We wish to call the common aglycone of **22** and **24**, that is $3\beta,7\beta,16\beta$-trihydroxycucurbita-5,24-diene (**26**), hydroxyhebevinogenin.

Hebevinoside V (**27**) was obtained as colorless needles from methanol, mp 165—166 °C, $[\alpha]_D^{20}$ $+83°$ ($c = 0.053$, CHCl$_3$), UV: end absorption, IR$_{max}^{KBr}$ cm^{-1}: 3420, 1743, 1725, 1640, 1087, 1032. The ^{1}H-NMR spectrum of **27** suggested that **27** is a methylated derivative of **22**, because the methoxyl group signal was observed at 3.34 ppm (3H, s), the signal of a hydrogen on the carbon bearing a methoxyl group was observed at 3.42 ppm (d, $J = 6.2$ Hz) and this hydrogen was coupled with an olefinic hydrogen at position 6 (5.75 ppm, d, $J = 6.2$ Hz). Furthermore, the methoxyl signal was observed at 56.3 ppm and that of C-7 was shifted to 77.8 ($+10.3$) ppm in the ^{13}C-NMR spectrum of **27** from 67.5 ppm in the case of **22** (see Table II). Thus, the structure of hebevinoside V was deduced to be **27** (see Chart 7), in which the aglycone is common with those of **1** and **16**, that is methoxyhebevinogenin (**21**).

It remains to be clarified whether the absolute structures of hebevinosides I, II, III, IV,

and V are expressible as **1**, **22**, **24**, **16**, and **27**, respectively.
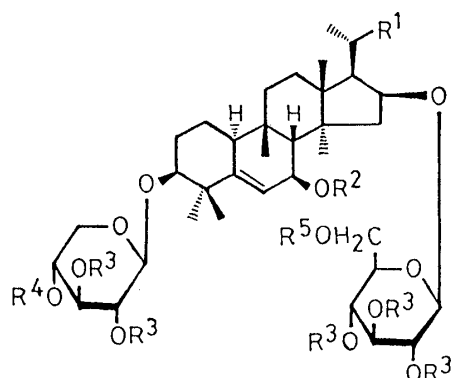
22: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = R^3 = H$, $R^4 = R^5 = Ac$
23: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = R^3 = R^4 = R^5 = Ac$
24: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = R^3 = R^4 = H$, $R^5 = Ac$
25: $R^1 = CH_2CH_2CH_2CH(CH_3)_2$, $R^2 = R^3 = R^4 = R^5 = Ac$
27: $R^1 = CH_2CH_2CH = C(CH_3)_2$, $R^2 = Me$, $R^3 = H$, $R^4 = R^5 = Ac$

Chart 7

On the intraperitoneal administration of 50 mg/kg of **1** or **27**, or 100 mg/kg of **22** or **24** to mice, five among five, three among four, four among five, or four among five mice died after paralysis within 72 h, respectively. However, **16** caused no paralytic death in mice at a dose of 200 mg/kg. This is the first isolation of these paralytic cucurbitane-type natural products from Basidiomycetes, to our knowledge. Whether these hebevinosides I, IV and V which have the 7β-methoxyl group are genuine or artifact metabolites will be reported in the following paper.

Experimental

All melting points were measured on a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. The optical rotations were measured with a JASCO DIP-181 digital polarimeter. The UV spectra were recorded with a Hitachi 323 recording spectrophotometer, the IR spectra with a Hitachi EPI-G3 grating infrared spectrophotometer, the EI-MS spectra with a JEOL JMS-01SG-2, a Hitachi M-60, or a Hitachi RMU-7M mass spectrometer, the FD-MS spectra with a JEOL JMS-01SG-2 mass spectrometer, the ^{1}H-NMR spectra with a JEOL JNM-GX270 FT-NMR, a JEOL JNM-FX270 FT-NMR spectrometer at 270 MHz, or a JEOL JNM-PS100 NMR spectrometer at 100 MHz and the ^{13}C-NMR spectra with a JEOL JNM-GX270 FT-NMR or a JEOL JNM-FX270 FT-NMR spectrometer at 67.8 MHz. Chemical shifts are expressed in δ (ppm) values from tetramethylsilane (TMS) as an internal standard. The following abbreviations are used; s, singlet, d: doublet t: triplet, q: quartet, dd: double doublet, dt: doublet of triplet, m: multiplet br: broad. The intensity data on X-ray crystallographic analysis were collected with a Rigaku automated four-circle diffractometer. Gas liquid chromatographic (GLC) analysis was carried out with a Shimadzu GC-5A gas chromatograph equipped with a hydrogen flame ionization detector. TLC analyses were carried out on silica gel plates (Merck Kieselgel 60 G or 60 GF$_{254}$). Column chromatographic separations were carried out on silica gel (Mallinckrodt Silic AR CC-7) or Sephadex LH-20 (Pharmacia). Preparative thin layer chromatographic (preparative TLC) separation was carried out on silica gel plates (Merck Kieselgel 60 HF$_{254}$). The toxicity of each hebevinoside was examined by intraperitoneal injection of 0.1 ml of a solution of each sample in dimethyl sulfoxide (DMSO) into each mouse (ddY, male, 20—25 g) and by observation of the behavior of the injected mice within 72 h (3—5 mice were employed for each sample).

Isolation of Hebevinosides——Details were reported previously.[1] From the aqueous methanolic extract (50.5 g) of dried fruit-bodies (277.6 g) obtained by cultivation of *H. vinosophyllum* on PDA (potato-dextrose-agar) medium[15] or MYA (malt extract-yeast extract-agar) medium,[1] 20.0 g of *n*-BuOH-soluble fraction was obtained. Repeated column chromatography of the *n*-BuOH-soluble fraction on silica gel and Sephadex LH-20 afforded **16** (56 mg), **27** (112 mg), **22** (250 mg), **1** (472 mg), and **24** (278 mg).

Hebevinoside I (**1**): Amorph. Hydrogenation of **1** (60 mg) with PtO$_2$ (30 mg) as the catalyst in EtOH (23 ml) for 1 h afforded **2** (60 mg), amorph., which was acetylated with Ac$_2$O (0.5 ml) in pyridine (1.0 ml) at room temp. overnight to give **3** (65 mg), *Anal.* Calcd for C$_{56}$H$_{86}$O$_{19}$: C, 63.28; H, 8.10. Found: C, 63.04; H, 8.03. UV: end absorption. IR$_{max}^{KBr}$ cm^{-1}: 1760, 1655, 1040. ^{1}H-NMR (CDCl$_3$): 0.88 (3H, d, $J = 6.3$ Hz), 0.89 (6H, d, $J = 6.6$ Hz), 1.99, 2.01, 2.03, 2.04, 2.06 (each 3H, s, Ac × 5), 2.02 (6H, s, Ac × 2).

Hebevinoside II (**22**): Amorph., ^{1}H-NMR (C$_5$D$_5$N): 0.88, 1.12, 1.17, 1.38, 1.61 (each 3H, s), 1.04 (3H, d, $J = 6.8$ Hz, H$_3$-21), 1.70, 1.73 (each 3H, s, H$_3$-26 and H$_3$-27), 1.96, 2.04 (each 3H, s, Ac), 4.42 (1H, br d, $J = 5.1$ Hz, H-7), 4.67, 4.82 (each 1H, d, $J = 7.7$ Hz, H-xyl. 1 and H-glc.1), 5.53 (1H, br t, $J = 7.7$ Hz, H-24), 6.12 (1H, d, $J = 5.1$ Hz, H-6). **22** (10 mg) was acetylated with Ac$_2$O (0.1 ml) in pyridine (0.2 ml) as usual to afford **23** (10 mg), amorph., ^{1}H-NMR

(CDCl$_3$): 1.99, 2.01, 2.04, 2.10 (each 3H, s, Ac × 4), 2.00, 2.02 (each 6H, s, Ac × 4).

Hebevinoside III (**24**): Amorph., ^{1}H-NMR (C$_5$D$_5$N): 0.88, 1.12, 1.18, 1.36, 1.61 (each 3H, s), 1.04 (3H, d, $J=$ 6.0 Hz, H$_3$-21), 1.69, 1.72 (each 3H, s, H$_3$-26 and H$_3$-27), 2.05 (3H, s, Ac), 4.41 (1H, br d, $J=5.6$ Hz, H-7), 4.67, 4.81 (each 1H, d, $J=7.7$ Hz, H-xyl.1 and H-glc.1), 5.52 (1H, br t, $J=6.0$ Hz, H-24), 6.12 (1H, d, $J=5.6$ Hz, H-6). **24** (25 mg) was acetylated with Ac$_2$O (0.1 ml) in pyridine (0.2 ml) as usual to afford a heptaacetate (30 mg), amorph., which was identical with **23** in terms of ^{1}H-NMR spectra (CDCl$_3$), IR spectra (KBr), and TLC behavior (solvent: CHCl$_3$–MeOH (50 : 1, v/v)). Catalytic hydrogenation of hebevinoside III heptaacetate (**23**) (20 mg) with PtO$_2$ (10 mg) in EtOH (8 ml) for 1 h gave **25** (20 mg), colorless needles from MeOH, ^{1}H-NMR (CDCl$_3$): 0.88 (3H, d, $J=6.6$ Hz), 0.89 (6H, d, $J=6.6$ Hz).

Hebevinoside IV (**16**): EI-MS m/z (%): 572 (19, M$^+$ − CH$_3$OH), 471 (10, M$^+$ − C$_5$H$_9$O$_4$), ^{1}H-NMR (CDCl$_3$): 0.69, 0.97, 1.00, 1.12, 1.22 (each 3H, s), 0.99 (3H, d, $J=8.1$ Hz, H$_3$-21), 1.62, 1.70 (each 3H, s, H$_3$-26 and H$_3$-27), 3.34 (3H, s, OCH$_3$), 3.42 (1H, br d, $J=5.6$ Hz, H-7), 3.98 (1H, dd, $J_1=11.8$, $J_2=4.5$ Hz, H-3), 4.36 (1H, d, $J=6.0$ Hz, H-xyl.1), 4.44 (1H, dd, $J_1=7.3$, $J_2=13.7$ Hz, H-16), 5.19 (1H, br t, $J=7.7$ Hz, H-24), 5.74 (1H, d, $J=5.6$ Hz, H-6). **16** (25 mg) was hydrogenated with PtO$_2$ (12.5 mg) in EtOH (7.5 ml) for 1 h to give **17** (25 mg), mp 194—195 °C, C$_{36}$H$_{62}$O$_7$ (EI-MS m/z (%): 606 (3, M$^+$)), ^{1}H-NMR (CDCl$_3$): 0.88 (6H, d, $J=7$ Hz), 0.97 (3H, d, $J=5$ Hz). In addition, **16** (25 mg) was acetylated with Ac$_2$O (0.1 ml) in pyridine (0.2 ml) as usual to afford **18** (25 mg), mp 129—130 °C, EI-MS m/z (%): 513 (30, M$^+$ − C$_{11}$H$_{15}$O$_7$), FD-MS m/z: 772 (M$^+$), ^{1}H-NMR (CDCl$_3$): 2.03 (6H, s, Ac × 2), 2.04, 2.05 (each 3H, s, Ac × 2). Hydrogenation of **18** (18 mg) with PtO$_2$ (9 mg) in EtOH (12.5 ml) for 1 h gave **19** (18 mg), C$_{44}$H$_{70}$O$_{11}$ (FD-MS m/z: 774 (M$^+$)), EI-MS m/z (%): 515 (39, M$^+$ − C$_{11}$H$_{15}$O$_7$), ^{1}H-NMR (CDCl$_3$): 0.87 (6H, d, $J=7$ Hz), 0.95 (3H, d, $J=5$ Hz).

Hebevinoside V (**27**): ^{1}H-NMR (CDCl$_3$): 0.68, 0.97, 1.01, 1.12, 1.22 (each 3H, s), 0.96 (3H, d, $J=5.6$ Hz, H$_3$-21), 1.62, 1.69 (each 3H, s, H$_3$-26 and H$_3$-27), 2.10 (6H, s, Ac × 2), 3.34 (3H, s, OMe), 3.42 (1H, br d, $J=6.2$ Hz, H-7), 4.07 (1H, dd, $J_1=12.0$, $J_2=4.3$ Hz, H$_a$-glc.6), 4.16 (1H, dd, partly overlapped with other signals, H$_b$-glc.6), 4.42 (2H, d, $J=6.4$ Hz, H-xyl.1 and H-glc.1), 4.81 (1H, dt, $J_1=J_2=7.7$, $J_3=5.0$ Hz, H-xyl.4), 5.14 (1H, br t, $J_1=J_2=$ 6.9 Hz, H-24), 5.75 (1H, d, $J=6.2$ Hz, H-6).

Methylation of 1——NaH (about 50% in Bayol 85) (120 mg) was stirred with DMSO (1.0 ml) at 65 °C for 1 h under an N$_2$ gas flow. A solution of **1** (30 mg) in DMSO (0.6 ml) was then added and the reaction mixture was stirred at room temp. for 30 min under an N$_2$ gas flow. CH$_3$I (0.5 ml) was added to the solution under ice-cooling and the reaction mixture was stirred at room temp. overnight.[2] After dilution with ice-water, the mixture was extracted with CHCl$_3$ and the organic phase was washed with water, dried over Na$_2$SO$_4$ and evaporated *in vacuo* to afford a resinous residue. The residue was chromatographed on a column of silica gel (4 g) with C$_6$H$_6$–CHCl$_3$ (1 : 1, v/v) to afford **4** (17 mg), amorph., ^{1}H-NMR (CDCl$_3$): no Ac, 3.28, 3.39, 3.45, 3.52, 3.54, 3.56, 3.60, 3.61 (each 3H, s, OMe × 8).

Methanolysis of 4——A solution of **4** (6.5 mg) in methanolic 2 N HCl (1.0 ml) was refluxed for 4 h under an N$_2$ gas flow. The reaction mixture was neutralized with Amberlite MB-3 and evaporated *in vacuo* to afford a resinous residue. It was examined by GLC (column, 5% neopentylglycol succinate polyester on Chromosorb W-HP, 80—100 mesh, 3 mm × 2.0 m; column temp., 155 °C; carrier gas, N$_2$ 40 ml/min) and shown to consist of methyl 2,3,4,-tri-*O*-methylxylopyranoside (retention time (t_R): 4.1, 5.1 min) and methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (t_R: 8.6, 12.1 min).

Acid Hydrolysis of 1——A solution of **1** (132 mg) in methanolic 2 N HCl (4.0 ml) was refluxed under an N$_2$ gas flow at 75 °C for 2 h. The reaction mixture was diluted with ice-water, and extracted with CHCl$_3$. The CHCl$_3$ layer was washed with water, dried over Na$_2$SO$_4$, and evaporated *in vacuo* to afford a product mixture (84 mg), which was chromatographed on a column of silica gel (8.4 g) with C$_6$H$_6$–CHCl$_3$ (1 : 1, v/v) and CHCl$_3$ to give **5** (yield after crystallization from acetone: 15.2 mg), EI-MS m/z (%): 472 (100, M$^+$), ^{1}H-NMR (CDCl$_3$): 0.73, 0.90, 0.98 (each 3H, s), 1.09, 1.15 (each 6H, s), 0.94 (3H, d, $J=7$ Hz, H$_3$-21), 2.07, 2.15 (each 1H, m, H$_2$-7), 2.37, 2.55 (each 1H, m, H$_2$-2), 3.15 (3H, s, OMe), 3.43 (1H, dd, $J_1=9$, $J_2=6$ Hz, H-3), 4.37 (1H, dd, $J_1=13$, $J_2=6$ Hz, H-16), 5.47 (1H, br s, $W_{1/2}=$ 8.1 Hz, H-1), 5.58 (1H, br s, $W_{1/2}=10.8$ Hz, H-6). The aqueous layer was neutralized with Dowex 2 × 8 (OH type) and lyophilized to afford a resinous residue. It was examined by TLC (solvent: CHCl$_3$–MeOH–H$_2$O (7 : 3 : 0.5, v/v), detection reagent: anisaldehyde–H$_2$SO$_4$) to show that the resinous residue consists of methyl xyloside (*Rf*: 0.53) and methyl glucoside (*Rf*: 0.33).

Acetylation of 5——Compound **5** (17 mg) was acetylated with Ac$_2$O (0.5 ml) in pyridine (1.0 ml) as usual to afford a product mixture (14.5 mg) which was chromatographed on a column of silica gel (1.5 g) with C$_6$H$_6$–CHCl$_3$ (20 : 1 and 1 : 1, v/v). The fraction eluted with C$_6$H$_6$–CHCl$_3$ (20 : 1) afforded **7** (7 mg), amorph., ^{1}H-NMR (CDCl$_3$): 2.01, 2.05 (each 3H, s, Ac × 2), 4.67 (1H, dd, $J_1=9.1$, $J_2=6.1$ Hz, H-3), 5.28 (1H, dd-like, $W_{1/2}=10.8$ Hz, H-16). The fraction eluted with C$_6$H$_6$–CHCl$_3$ (1 : 1) afforded **6** (4.5 mg), amorph., ^{1}H-NMR (CDCl$_3$): 2.05 (3H, s, Ac), 4.39 (1H, dd, $J_1=13$, $J_2=6$ Hz, H-16), 4.67 (1H, dd, $J_1=9.5$, $J_2=6.1$ Hz, H-3).

Oxidation of 6——A solution of **6** (15 mg) in pyridine (0.13 ml) was added to a complex prepared with CrO$_3$ (25 mg) and pyridine (0.25 ml) under ice-cooling, and the reaction mixture was stirred under ice-cooling for 1 h. After addition of a few drops of MeOH, the mixture was diluted with water and extracted with CHCl$_3$. The CHCl$_3$ layer was washed with water, dried over Na$_2$SO$_4$, and evaporated *in vacuo* to afford a product mixture (14 mg), which was chromatographed on a column of silica gel (2.8 g) with C$_6$H$_6$–CHCl$_3$ (5 : 1, v/v) to give **8** (5 mg), amorph., ^{1}H-NMR

(CDCl$_3$): 0.87 (3H, d, $J = 6.6$ Hz, H$_3$-21), 1.61 (1H, m, H-20), 1.99 (3H, s, Ac), 2.14 (1H, d, $J = 8.6$ Hz, H-17), 3.10 (3H, s, OMe), 4.62 (1H, dd, $J_1 = 10.8$, $J_2 = 5.4$ Hz, H-3), 5.47 and 5.54 (each 1H, t-like, $W_{1/2} = 10.8$ Hz, H-1 and H-6, respectively), ^{13}C-NMR (CDCl$_3$): 21.0 (q, CO$\underline{C}$H$_3$), 60.8 (q, OMe), 74.8 (s, C-25), 75.7 (d, C-3), 114.1, 120.2 (each d, C-1 and C-6), 139.2, 140.5 (each s, C-5 and C-10), 170.8 (s, $\underline{C}$OCH$_3$), 220.2 (s, C-16).

Acid Hydrolysis of 2——A solution of **2** (942 mg) in methanolic 2 N HCl (5.0 ml) was refluxed under an N$_2$ gas flow at 70 °C for 2 h. The reaction mixture was treated in the same way as for acid hydrolysis of **1** to afford a product mixture (635 mg), which was chromatographed on a column of silica gel (50 g) with C$_6$H$_6$–CHCl$_3$ (1 : 1, v/v) to give crude **9** (159 mg). After crystallization from acetone, 20 mg of crude **9** afforded 9 mg of pure **9**, colorless needles, ^{1}H-NMR (CDCl$_3$): 0.73, 0.89, 0.98, 1.08, 1.09 (each 3H, s), 0.86 (6H, d, $J = 6.6$ Hz), 0.94 (3H, d, $J = 6.3$ Hz), 2.10, 2.23 (each 1H, m, H$_2$-7), 2.44, 2.50 (each 1H, m, H$_2$-2), 3.45 (1H, dd, $J_1 = 9.0$, $J_2 = 5.4$ Hz, H-3), 4.41 (1H, dd, $J_1 = 14.4$, $J_2 = 7.2$ Hz, H-16), 5.49 (1H, br s, $W_{1/2} = 9.4$ Hz, H-1), 5.61 (1H, br s, $W_{1/2} = 10.1$ Hz, H-6), ^{13}C-NMR (CDCl$_3$): 72.9 (d, C-16), 73.8 (d, C-3), 113.5, 120.7 (each d, C-1 and C-6), 139.9, 140.4 (each s, C-5 and C-10).

Acetylation of 9——Compound **9** (8 mg) was acetylated with Ac$_2$O (0.1 ml) in pyridine (0.3 ml) at room temp. overnight to afford a product mixture (8 mg), which was separated by preparative TLC (solvent for development: C$_6$H$_6$–CHCl$_3$ (1 : 1, v/v), solvent for extraction: CHCl$_3$–acetone (1 : 1, v/v)) to afford **10** (1 mg), amorph., IR$_{max}^{KBr}$ cm^{-1}: 3500, 1740, ^{1}H-NMR (CDCl$_3$): 2.04 (3H, s, Ac), 4.40 (1H, dd, H-16), 4.66 (1H, dd, H-3), and **11** (2 mg), amorph., ^{1}H-NMR (CDCl$_3$): 2.01, 2.05 (each 3H, s, Ac × 2), 4.66 (1H, dd, H-3), 5.28 (1H, dd, H-16).

Desacetylation of 1——A solution of **1** (40 mg) in methanolic 0.1% KOH was stirred at room temp. for 40 min. The reaction mixture was neutralized with Amberlite MB-3 and evaporated *in vacuo* to afford a product mixture (40 mg), which was purified on a column of silica gel (3 g) with C$_6$H$_6$–acetone (5 : 4, v/v) to give **12** (13 mg), amorph., ^{1}H-NMR (C$_5$D$_5$N): 0.74, 1.09, 1.16, 1.24, 1.63 (each 3H, s), 1.69, 1.73 (each 3H, s, H$_3$-26 and H$_3$-27), 3.21 (3H, s, OMe), 3.41 (1H, d, $J = 5.4$ Hz, H-7), 4.79 (2H, d, $J = 7.6$ Hz, H-xyl.1 and H-glc.1), 5.55 (1H, t, $J_1 = J_2 = 5.0$ Hz, H-24), 5.88 (1H, d, $J = 5.4$ Hz, H-6), ^{13}C-NMR (C$_5$D$_5$N): 56.2 (q, OMe), 77.6 (d, C-7), 82.1 (d, C-16), 87.4 (d, C-3), 119.4 (d, C-6), 126.8 (d, C-24), 130.0 (s, C-25), 147.7 (s, C-5), 107.7, 74.9, 78.5, 71.1, 67.0 (C-xyl.1, 2, 3, 4, and 5, respectively), 106.8, 75.6, 78.7, 72.0, 78.2, 63.2 (C-glc.1, 2, 3, 4, 5, and 6, respectively).

EI-MS Analysis of Pertrimethylsilylether of 1——A 1 mg portion of **1** in a microtest tube with a stopper was shaken vigorously with 0.2 ml of dry pyridine, 0.1 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane at room temp. for 10 min.[16] The reaction mixture was treated with an N$_2$ gas flow to remove the solvent. The solid residue obtained was extracted with acetone. After removing the acetone with an N$_2$ gas flow, the product residue obtained was immediately analyzed with a JEOL JMS-01SG-2 mass spectrometer.

Enzymatic Hydrolysis of 12——A suspension of **12** (40 mg) and Molsin (Seishin Pharm. Co.)[6] (80 mg) in EtOH (2.5 ml) and 0.1 M citric acid–0.2 M Na$_2$HPO$_4$ buffer solution (pH 4.0) (7.5 ml) was stirred at 37 °C for 96 h. The reaction mixture was treated with an N$_2$ gas flow at room temp. to remove EtOH and the residue was extracted with CHCl$_3$. The CHCl$_3$ layer was washed with water, dried over Na$_2$SO$_4$ and evaporated *in vacuo* to afford a product mixture (25 mg), which was chromatographed on a column of silica gel (1 g) with C$_6$H$_6$–acetone (3 : 1, v/v) to give **13** (4.5 mg), amorph., ^{1}H-NMR (C$_5$D$_5$N): 1.15 (3H, t, $J_1 = J_2 = 7.4$ Hz, OCH$_2$C$\underline{H}$$_3$), 3.45, 3.56 (each 1H, dd, $J_1 = 15.8$, $J_2 = 7.4$ Hz, OC$\underline{H}$$_2CH_3$), 3.62 (1H, d, $J = 5.0$ Hz, H-7), 4.79 (1H, d, $J = 7.9$ Hz, H-glc.1), 5.55 (1H, t, $J_1 = J_2 = 5.0$ Hz, H-24), 5.98 (1H, d, $J = 5.0$ Hz, H-6), ^{13}C-NMR (C$_5$D$_5$N): 16.0 (q, OCH$_2$$\underline{C}H_3$), 63.2 (t, O$\underline{C}H_2CH_3$), 75.5 (d, C-3), 76.1 (d, C-7), 82.1 (d, C-16), 120.5 (d, C-6), 126.8 (d, C-24), 130.0 (s, C-25), 147.4 (s, C-5), 106.7, 75.5, 78.1, 72.0, 78.6, and 63.7 (C-glc.1, 2, 3, 4, 5, and 6, respectively).

Enzymatic Hydrolysis of 16——A suspension of **16** (75 mg) and Molsin[6] (400 mg) in EtOH (18 ml) and 0.1 M citric acid–0.2 M Na$_2$HPO$_4$ buffer solution (pH 4.0) (18 ml) was stirred at 37 °C for 96 h. The reaction mixture was treated in the same way as for enzymatic hydrolysis of **12** to afford a product mixture (50 mg), which was chromatographed on a column of silica gel (2.5 g) with C$_6$H$_6$–CHCl$_3$ (1 : 2, v/v) to give **20** (yield after crystallization from C$_6$H$_6$: 5 mg), ^{1}H-NMR (CDCl$_3$): 1.13 (3H, t, $J_1 = J_2 = 6.8$ Hz, OCH$_2$C$\underline{H}$$_3$), 3.33, 3.55 (each 1H, dd, $J_1 = 9.2$, $J_2 = 6.8$ Hz, OC$\underline{H}$$_2CH_3$), 3.43 (1H, t, $J_1 = 3.2$, $J_2 = 2.1$ Hz, H-3), 3.46 (1H, br d, $J = 5.2$ Hz, H-7), 4.36 (1H, dd, $J_1 = 13.5$, $J_2 = 7.3$ Hz, H-16), 5.12 (1H, t, $J_1 = J_2 = 6.8$ Hz, H-24), 5.72 (1H, d, $J = 5.2$ Hz, H-6), ^{13}C-NMR (CDCl$_3$): 14.7 (q, OCH$_2$$\underline{C}H_3$), 62.7 (t, O$\underline{C}H_2CH_3$), 71.6 (d, C-16), 74.1 (d, C-3), 75.8 (d, C-7), 120.8 (d, C-6), 123.7 (d, C-24), 131.5 (s, C-25), 145.2 (s, C-5).

Methylation of 24——NaH (about 50% in Bayol 85) (120 mg) was stirred with DMSO (1.0 ml) at 65 °C for 1 h under an N$_2$ gas flow. A solution of **24** (25 mg) in DMSO (0.6 ml) was added to this reagent and the reaction mixture was stirred at room temp. for 30 min under an N$_2$ gas flow. CH$_3$I (0.75 ml) was added to the solution under ice-cooling and the reaction mixture was stirred at room temp. overnight.[2] After dilution with ice-water, the mixture was treated in the same way as for methylation of **1** to afford a resinous residue. The residue was chromatographed on a column of silica gel (4.0 g) with CHCl$_3$–C$_6$H$_6$ (4 : 1, v/v) to give the permethylether (12 mg), which was identical with **4** in terms of IR spectra (KBr) and TLC behavior (solvent: CHCl$_3$–MeOH (50 : 1, v/v)).

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