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On the Diterpenoids of *Andrographis paniculata*: X-Ray Crystallographic Analysis of Andrographolide and Structure Determination of New Minor Diterpenoids[1)]

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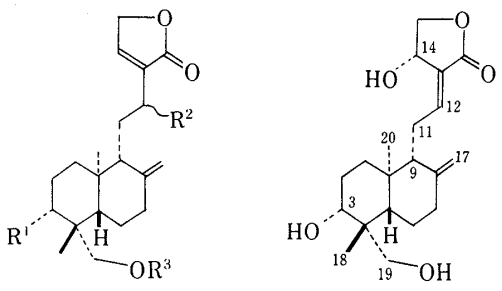
The crystal structure of andrographolide (**8**) was determined and the absolute configuration at C-14, which was previously undecided, was established as *R*. Reexamination of the constituents of *Andrographis paniculata* NEES resulted in the isolation of three new diterpenoids of *ent*-labdane type, andrographanin, andropanoside, and 14-deoxy-12-methoxy-andrographolide, together with three known compounds, 14-deoxyandrographolide (**4**), neoandrographolide (**5**) and andrographolide (**8**). The structures of the new diterpenoids were determined to be **1**, **2**, and **3** on the basis of spectral and chemical evidence.

Keywords——*Andrographis paniculata*; Acanthaceae; andrographolide; X-ray analysis; andrograpanin; andropanoside; 14-deoxy-12-methoxy-andrographolide; *ent*-labdane diterpenoid; structure elucidation

In southeast Asia, *Andrographis paniculata* NEES (Acanthaceae) is widely used in traditional medicine as an antidote against poisons of snakes and insects, and as an antimalarial agent. This plant is very bitter, and andrographolide (**8**)[2)] has already been isolated as the main bitter constituent, together with 14-deoxyandrographolide (**4**),[3)] neoandrographolide (**5**),[4)] 14-deoxy-11,12-didehydroandrographolide (**9**),[3)] and 14-deoxy-11-oxoandrographolide (**10**).[3)] In order to determine the absolute configuration at C-14 of andrographolide (**8**) and to investigate the minor diterpenoids, we reexamined the constituents of this plant and isolated three new diterpenoids, andrograpanin (**1**), andropanoside (**2**), and 14-deoxy-12-methoxy-andrographolide (**3**), together with three known compounds, **4**, **5**, and **8**. This report describes the results.

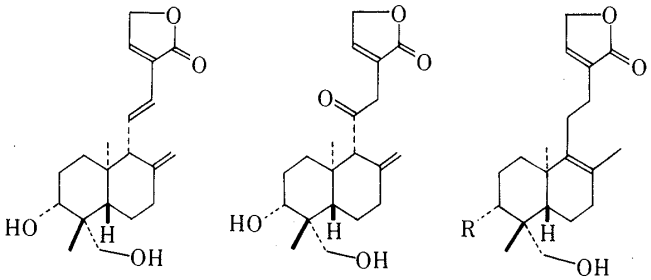
The structure of andrographolide (**8**) has been extensively studied and the absolute stereostructure, except for that of C-14, has already been established as **8**. The failure of various chemical and spectral attempts to determine the complete stereochemistry led us to undertake an X-ray analysis. The molecular structure of **8** is illustrated in Fig. 1, while bond distances and angles involving non-hydrogen atoms are given in Table I. Since the other part of **8** had been established as having *ent*-labdane structure, the absolute stereochemistry at C-14 was established as *R*.

One of the new diterpenoids, andrograpanin (**1**), $C_{20}H_{30}O_3$, mp 104—106 °C, showed infrared (IR) bands at 3630 (hydroxy group), 1760 (α,β-unsaturated-γ-lactone), 1639 (double bond), and 886 (*exo*-methylene) cm^{-1}. The ^1H-nuclear magnetic resonance (NMR) spectrum showed signals at δ 3.34 and 3.72 (each 1H, ABd, $J=11$ Hz), besides singlets due to two



1 : $R^1=R^2=R^3=H$
2 : $R^1=OH$; $R^2=H$; $R^3=\beta$-D-Gluc
3 : $R^1=OH$; $R^2=OMe$; $R^3=H$
4 : $R^1=OH$; $R^2=R^3=H$
5 : $R^1=R^2=H$; $R^3=\beta$-D-Gluc
6 : $R^1=R^2=H$; $R^3=Ac$
7 : $R^1=OAc$; $R^2=H$; $R^3=\beta$-D-$GlucAc_4$

Gluc=glucopyranoside



9 10 11 : R=H 12 : R=OH

Chart 1

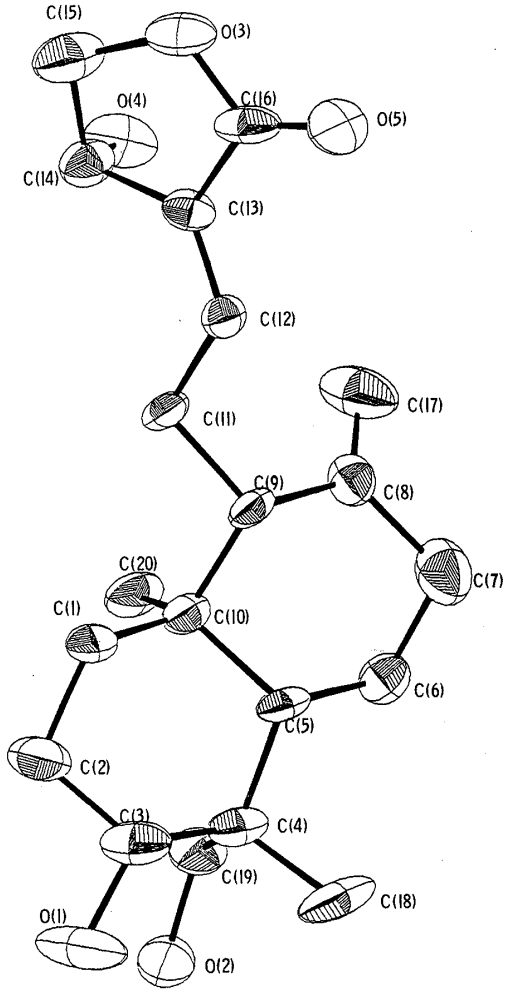


Fig. 1. Molecular Structure of Andrographolide (**8**)

tertiary methyl groups at δ 0.65 and 0.97 and singlets (each 1H) assigned to protons of *exo*-methylene at δ 4.53 and 4.82. The AB doublets were assigned to the protons of a hydroxymethyl group adjacent to a quaternary carbon since the signals were shifted downfield to δ 3.78 and 4.17 in the ^{1}H-NMR spectrum of the acetate (**6**), $C_{22}H_{32}O_4$. The ^{1}H-NMR spectrum of **1** further showed a signal at δ 4.72 (2H, m) assigned to a methylene group attached to oxygen of the lactone group and a signal at δ 7.10 (1H, m, $W_{1/2}=4$ Hz) due to an olefinic proton conjugated with a lactone. Upon irradiation of these signals, the other signals changed to a broad singlet. This finding showed that andrograpanin (**1**) has the same α,β-unsaturated butenolide as 14-deoxyandrographolide (**4**) in the α,β-unsaturated γ-lactone portion. From these results, andrograpanin (**1**) is tricyclic. On the basis of the structures of diterpenoids isolated so far from the plant and the analysis of the ^{13}C-NMR spectrum of **1** (Table II), andrograpanin (**1**) was presumed to have a structure corresponding to the aglucone of neoandrographolide (**5**) except for the stereochemistry. This presumption was verified by the finding that isoandrograpanin (**11**), $C_{20}H_{30}O_3$, obtained by acid treatment of **1** was identical with the isoaglucone of neoandrographolide (**5**), obtained by acid hydrolysis. The remaining problem was the stereochemistry at C-9, which was determined from the fact that andrograpanin (**1**) was identical with the genuine aglucone of **5** obtained by hydrolysis with takadiastase.[5] Accordingly, andrograpanin has the structure **1**.

The second new compound, andropanoside (**2**),[6] $C_{26}H_{40}O_9$, mp 199—202 °C, showed a

TABLE I. Bond Distances (Å) and Angles (°) of **8** Involving Non-hydrogen Atoms with Their Estimated Standard Deviations in Parentheses

O(1)	– C(3)	1.455 (8)	O(2)	– C(19)	1.451 (7)		
O(3)	– C(15)	1.471 (8)	O(3)	– C(16)	1.341 (6)		
O(4)	– C(14)	1.426 (8)	O(5)	– C(16)	1.203 (7)		
C(1)	– C(2)	1.539 (9)	C(1)	– C(10)	1.507 (9)		
C(2)	– C(3)	1.525 (9)	C(3)	– C(4)	1.544 (9)		
C(4)	– C(5)	1.582 (8)	C(4)	– C(18)	1.537 (8)		
C(4)	– C(19)	1.536 (8)	C(5)	– C(6)	1.502 (9)		
C(5)	– C(10)	1.575 (7)	C(6)	– C(7)	1.578 (10)		
C(7)	– C(8)	1.528 (9)	C(8)	– C(9)	1.485 (9)		
C(8)	– C(17)	1.292 (9)	C(9)	– C(10)	1.566 (8)		
C(9)	– C(11)	1.577 (7)	C(10)	– C(20)	1.547 (8)		
C(11)	– C(12)	1.476 (8)	C(12)	– C(13)	1.352 (7)		
C(13)	– C(14)	1.507 (8)	C(13)	– C(16)	1.459 (8)		
C(14)	– C(15)	1.516 (8)					
C(15)	– O(3) – C(16)	110.5 (5)	C(2)	– C(1) – C(10)	112.5 (5)		
C(1)	– C(2) – C(3)	108.9 (5)	O(1)	– C(3) – C(2)	107.6 (5)		
O(1)	– C(3) – C(4)	111.4 (5)	C(2)	– C(3) – C(4)	113.7 (5)		
C(3)	– C(4) – C(5)	106.9 (5)	C(3)	– C(4) – C(18)	108.3 (5)		
C(3)	– C(4) – C(19)	112.4 (5)	C(5)	– C(4) – C(18)	107.6 (5)		
C(5)	– C(4) – C(19)	112.0 (5)	C(18)	– C(4) – C(19)	109.4 (5)		
C(4)	– C(5) – C(6)	112.7 (5)	C(4)	– C(5) – C(10)	116.2 (5)		
C(6)	– C(5) – C(10)	112.6 (5)	C(5)	– C(6) – C(7)	110.7 (5)		
C(6)	– C(7) – C(8)	108.4 (5)	C(7)	– C(8) – C(9)	112.1 (5)		
C(7)	– C(8) – C(17)	120.8 (6)	C(9)	– C(8) – C(17)	127.1 (6)		
C(8)	– C(9) – C(10)	112.1 (5)	C(8)	– C(9) – C(11)	113.1 (5)		
C(10)	– C(9) – C(11)	111.9 (4)	C(1)	– C(10) – C(5)	108.6 (5)		
C(1)	– C(10) – C(9)	110.3 (5)	C(1)	– C(10) – C(20)	111.6 (5)		
C(5)	– C(10) – C(9)	104.9 (4)	C(5)	– C(10) – C(20)	113.5 (5)		
C(9)	– C(10) – C(20)	107.7 (5)	C(9)	– C(11) – C(12)	111.7 (5)		
C(11)	– C(12) – C(13)	125.5 (5)	C(12)	– C(13) – C(14)	129.0 (5)		
C(12)	– C(13) – C(16)	121.2 (5)	C(14)	– C(13) – C(16)	109.7 (5)		
O(4)	– C(14) – C(13)	110.1 (5)	O(4)	– C(14) – C(15)	109.8 (5)		
C(13)	– C(14) – C(15)	100.3 (5)	O(3)	– C(15) – C(14)	106.6 (5)		
O(3)	– C(16) – O(5)	121.8 (5)	O(3)	– C(16) – C(13)	108.2 (5)		
O(5)	– C(16) – C(13)	129.9 (5)	O(2)	– C(19) – C(4)	112.6 (5)		

strong hydroxy group band at 3410—3240 cm^{-1} and bands at 1746 (α,β-unsaturated-γ-lactone), 1635 (double bond), and 900 (*exo*-methylene) cm^{-1} in the IR spectrum. Acid hydrolysis of **2** gave the isoaglucone (**12**), $C_{20}H_{30}O_4$, from the organic phase; the aqueous fraction showed a spot corresponding to D-glucose on paper partition chromatography. Accordingly, andropanoside (**2**) is a diterpenoid glucoside. The IR spectrum of the isoaglucone (**12**) showed bands at 3620 (hydroxy), 1760 (α,β-unsaturated-γ-lactone), and 1647 (double bond) cm^{-1} but no band at 900 cm^{-1} (*exo*-methylene) as was observed in the IR spectrum of andropanoside (**2**). This fact suggests that the *exo*-methylene had shifted to the endocyclic position during acid hydrolysis. This presumption was supported by the observation of a new signal (3H, s, vinyl methyl group) at δ 1.58 in the ^{1}H-NMR spectrum of **12**. The ^{1}H-NMR spectrum also showed a signal due to an axial proton attached to a carbon bearing a hydroxy group at δ 3.42 (1H, t, $J=8$ Hz) and signals assigned to a hydroxymethyl group at δ 3.28 and 4.18 (each 1H, ABd, $J=11$ Hz). These data suggest that the compound (**12**) is a double bond isomer of 14-deoxyandrographolide (**4**). In fact, an isomer obtained by acid treatment of **4** was identical with the isoaglucone (**12**) of andropanoside (**2**). Hydrolysis

TABLE II. Carbon-13 Chemical Shifts (δ)[a] of Andrograpanin (**1**), Andropanoside (**2**), 14-Deoxyandrographolide (**4**), Neoandrographolide (**5**), and Andrographolide (**8**)

Carbon	1[b]	2[c]	4[b]	5[c]	8[c]
1	39.2	37.9	37.0	39.2	37.6
2	19.1	29.2	28.3	19.5	29.1
3	35.6	79.3	80.6	36.6	80.1
4	39.7	43.4	42.9	39.9	43.4
5	56.5	55.9	55.5	56.4	55.6
6	24.6	25.1	24.1	24.9	24.5
7	38.7	38.9	38.3	38.9	38.3
8	147.8	148.3	147.1	148.4	148.1
9	56.7	56.8	56.3	56.9	56.6
10	39.0	39.8	39.1	38.7	39.4
11	21.9	22.5	22.1	22.3	25.1
12	24.7	25.3	24.6	25.1	146.8
13	135.0	134.4	134.8	134.4	130.2
14	144.1	145.4	144.3	145.4	66.2
15	70.2	70.6	70.2	70.6	75.2
16	174.5	174.6	174.5	174.7	170.5
17	106.9	107.1	107.4	107.0	108.7
18	27.2	24.4	22.8	28.3	23.7
19	65.1	72.0	64.3	72.7	64.2
20	15.3	15.0	15.3	15.5	15.3
1′		105.4		105.5	
2′		74.8		75.4	
3′		78.7		78.8	
4′		71.8		72.0	
5′		78.4		78.3	
6′		62.9		63.1	

a) The values were recorded at 50 MHz and are given in ppm from tetramethylsilane.
b) Measured for $CDCl_3$ solution.
c) Measured for C_5D_5N solution.

of andropanoside (**2**) with β-glucosidase gave 14-deoxyandrographolide (**4**) as the genuine aglucone. Accordingly, andropanoside (**2**) should be the 3- or 19-*O*-glucoside of **4**. Acetylation of **2** gave the pentaacetate (**7**), the ^{1}H-NMR spectrum of which showed signals due to an oxygenated methyl group at δ 3.55 and 3.95 (each 1H, ABd, $J=11$ Hz). Since these signals apparently did not undergo downfield shifts on acetylation, it was presumed that glucose was linked to C-19. This presumption was supported by comparisons of the ^{13}C-NMR spectra of andropanoside (**2**) and 14-deoxyandrographolide (**4**), in which the signals due to C-19 were found at δ 72.0 and 64.3, respectively (see Table II). The configuration of the glycosidic linkage was determined as β by comparing the ^{13}C-NMR spectral signals of the anomeric carbons (in C_5D_5N) of andropanoside (**2**) (δ 105.4), β-methyl-D-glucopyranoside (δ 105.2), and α-methyl-D-glucopyranoside (δ 101.3). Accordingly, the structure of andropanoside should be represented as **2**.

14-Deoxy-12-methoxy-andrographolide (**3**), $C_{21}H_{32}O_5$, mp 121—124 °C, showed ^{1}H-NMR signals due to two tertiary methyl groups at δ 0.61 and 1.24 and a signal assigned to an olefinic proton [δ 7.31 (1H, m, $W_{1/2}=4$ Hz)] on the α,β-unsaturated butenolide ring, like those of the above two compounds, **1** and **2**. The ^{1}H-NMR spectrum further showed a signal due to a methoxy group at δ 3.27 (3H, s), signals assigned to an oxygenated methyl group at δ 3.48 and 4.16 (each 1H, ABd, $J=11$ Hz), and signals arising from two secondary carbinyl protons at δ 3.46 (1H, br t, $J=9$ Hz) and 4.07 (1H, br t, $J=8$ Hz). These data, coupled with the

structures and spectral data of the diterpenoids isolated so far from the same plant, suggest that this compound (**3**) has an *ent*-labdane skeleton with two equatorial hydroxy groups at C-3 and C-19. The methoxy group was considered to be located at C-12 (an allylic position) since a signal due to a proton attached to carbon having a methoxy group appeared at a rather downfield position (δ 4.07). The supposed structure **3** could be explained reasonably in terms of an allylic rearrangement of andrographolide (**8**). The small amount of the sample prevented further characterization. However, the fragmentation pattern (Fig. 2) in the mass spectrum was consistent with the above structure. Namely, an ion at *m/z* 237, fragment ion B, supports

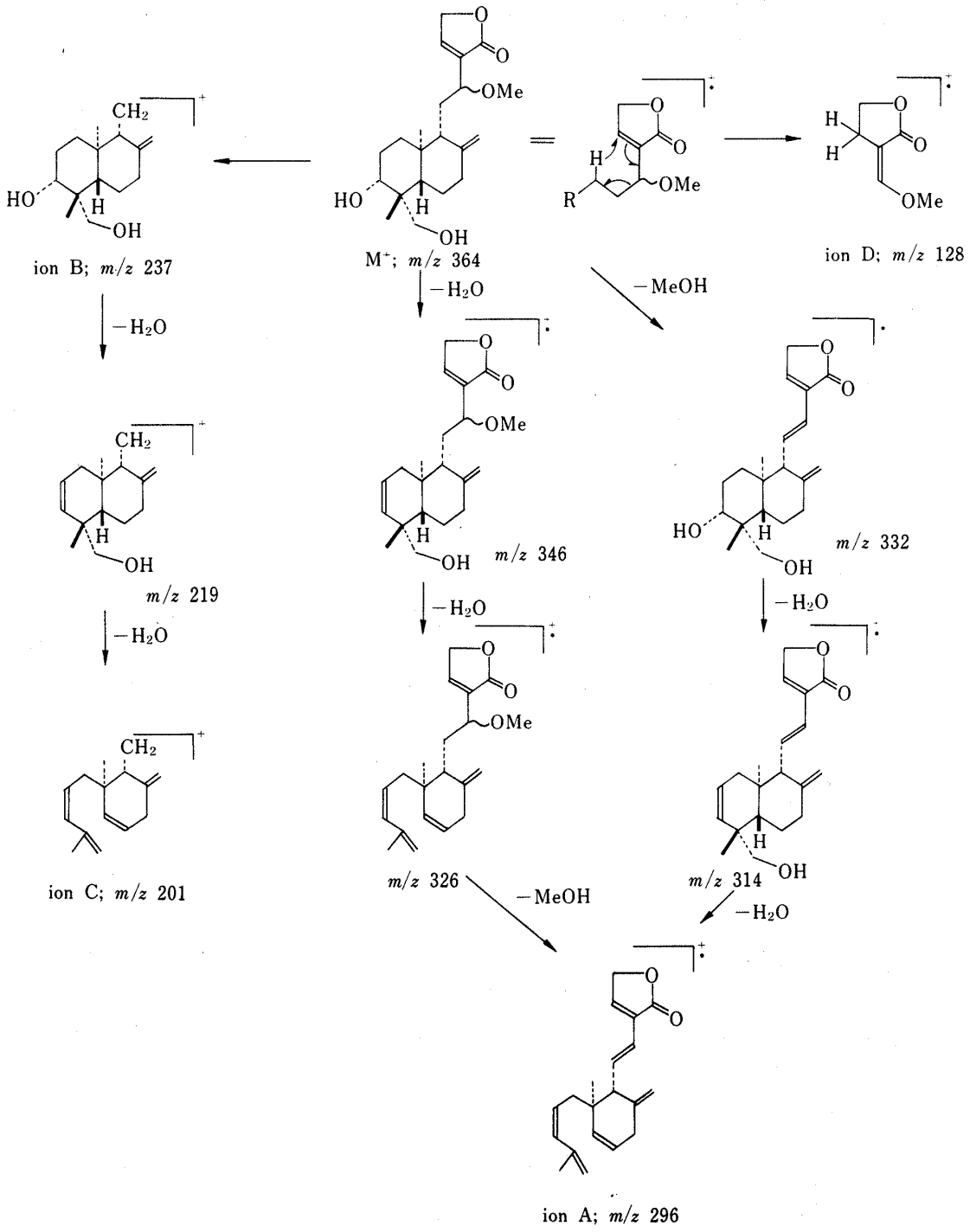


Fig. 2. Hypothetical Mechanism of Electron Impact Fragmentation of 14-Deoxy-12-methoxy-andrographolide (**3**)

TABLE III. Precise Mass Measurements of Fragment Ions A—D

	Found (±0.005 m.u.)	Calcd
Ion A, $C_{20}H_{24}O_2$	296.174	296.178
Ion B, $C_{15}H_{25}O_2$	237.185	237.185
Ion C, $C_{15}H_{21}$	201.164	201.164
Ion D, $C_6H_8O_3$	128.047	128.043

TABLE IV. Atomic Coordinates (× 10^4) and Thermal Parameters for Non-hydrogen Atoms of **8**, with Their e.s.d.'s in Parentheses

	x	y	z	B_{eq} [a]
O(1)	13800 (8)	−3569 (7)	4902 (3)	6.1
O(2)	16145 (6)	−856 (6)	4595 (2)	4.4
O(3)	3821 (6)	1385 (6)	−847 (2)	4.1
O(4)	8476 (6)	2447 (6)	−472 (2)	4.4
O(5)	2504 (6)	1856 (6)	219 (2)	4.2
C(1)	10811 (10)	−2018 (8)	2505 (3)	3.4
C(2)	12517 (10)	−2940 (9)	3015 (4)	4.6
C(3)	12280 (10)	−2571 (9)	3835 (3)	4.2
C(4)	12475 (9)	−698 (8)	4040 (3)	3.0
C(5)	10876 (8)	270 (8)	3460 (3)	2.8
C(6)	10855 (10)	2118 (9)	3609 (3)	4.2
C(7)	8922 (11)	2965 (9)	3144 (4)	5.1
C(8)	8932 (8)	2533 (8)	2314 (3)	3.2
C(9)	8952 (8)	699 (8)	2185 (3)	2.4
C(10)	10928 (9)	−148 (8)	2604 (3)	2.7
C(11)	8596 (9)	213 (8)	1326 (3)	3.1
C(12)	6562 (8)	773 (8)	963 (3)	2.9
C(13)	6056 (8)	1053 (8)	218 (3)	2.9
C(14)	7384 (8)	922 (9)	−408 (3)	3.3
C(15)	5741 (10)	724 (11)	−1078 (3)	4.7
C(16)	3948 (8)	1456 (8)	−95 (3)	3.0
C(17)	8926 (11)	3697 (9)	1813 (4)	5.5
C(18)	11817 (10)	−459 (11)	4827 (3)	4.9
C(19)	14678 (9)	−40 (8)	4037 (3)	3.6
C(20)	12814 (8)	595 (10)	2281 (3)	4.0

a) Equivalent isotropic thermal parameters were calculated from the refined anisotropic thermal parameters.

the proposed structure of ring A and an ion at m/z 128, fragment ion D, gives evidence for the position of the methoxy group. Thus, the structure of 14-deoxy-12-methoxy-andrographolide could be represented as **3**. However, the possibility that **3** is an artefact could not be excluded.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded with a Hitachi EPI-S2 or a Hitachi EPI-G2 spectrophotometer. ^{1}H-NMR spectra were recorded with a JEOL PS-100 spectrometer and ^{13}C-NMR spectra were obtained with a Bruker WH-200 spectrometer. Chemical shifts are given in δ values using tetramethylsilane as an internal standard. Ultraviolet (UV) spectra were taken with a Hitachi UV 200 double-beam spectrophotometer or a Union Giken SM-302 spectrophotometer. Optical rotations were measured with a Yanagimoto OR-50 or a Union Giken PM 201 instrument.

Mass spectra were determined with a JEOL JMS-01SG or a JEOL D-300 spectrometer. Kieselgel G (0.05—0.2 mm; Merck) or silicic acid (100 mesh, Mallinckrodt) was used for column chromatography and Kiesel gel GF_{254} plates (0.25 mm in thickness; Merck) were used for thin layer chromatography.

Isolation Procedures——Dried leaves (6.1 kg) of *Andrographis paniculata* NEES (collected in Sulawesi island, Indonesia) were extracted with MeOH (190 l) for 4 d at room temperature. The extract was concentrated *in vacuo* to about 8 l, and active charcoal (330 g) was added. After a day, the charcoal was filtered off and the filtrate was concentrated *in vacuo*. The resulting crystals (Cryst. I) were collected by filtration. The recovered charcoal was refluxed with MeOH (2 l) for 2 h. After removal of the charcoal by filtration, the filtrate was concentrated *in vacuo* to about 0.6 l and the resulting crystals (Cryst. II) were collected by filtration. Crude crystals (Cryst. I and II) were combined and recrystallized from MeOH to give andrographolide (**8**) (25.2 g). The filtrates obtained when the crude crystals (Cryst. I and II) were collected were combined and evaporated *in vacuo*. The residue was dissolved in EtOAc and the solution was washed with 5% Na_2CO_3 aqueous solution, H_2O, 1% HCl aqueous solution, and H_2O, successively. The dried EtOAc solution was concentrated *in vacuo* to give a residue (108.2 g), which was combined with the mother liquor of andrographolide (**8**). When the combined residue (200.1 g) was chromatographed on a silica gel (2.9 kg) column with $CHCl_3$–MeOH as the eluent with increasing MeOH content, andrograpanin (**1**), 14-deoxyandrographolide (**4**), 14-deoxy-12-methoxy-andrographolide (**3**), andrographolide (**8**), neoandrographolide (**5**), and andropanoside (**2**) were eluted succesively. The yields were **1** (0.03%), **2** (0.03%), **3** (0.001%), **4** (0.15%), **5** (0.05%), and **8** (0.6%). The physical data for the isolated compounds are as follows:

Andrograpanin (**1**): colorless needles, mp 104—106 °C (from Me_2CO–hexane), $[\alpha]_D^{23}$ −35.0° ($c=1.02$, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε): end absorption, 220 (4567): IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630, 1760, 1639, and 886; ^{1}H-NMR ($CDCl_3$) δ: 0.65 and 0.97 (each 3H, s, 2 Me), 3.34 and 3.72 (each 1H, ABd, $J=11$ Hz, 19-H_2), 4.53 (1H, s, 17-H_1), 4.72 (2H, m, 15-H_2), 4.82 (1H, s, 17-H_1), and 7.10 (1H, m, $W_{1/2}=4$ Hz, 14-H). *Anal.* Calcd for $C_{20}H_{30}O_3$: C, 75.47; H, 9.43. Found: C, 75.40; H, 9.43.

Andropanoside (**2**): colorless needles, mp 199—202 °C (from MeOH–Me_2CO), $[\alpha]_D^{23}$ −35.1 : ° ($c=0.57$, C_5H_5N); UV λ_{max}^{MeOH} nm (ε): end absorption, 220 (5301); IR ν_{max}^{KBr} cm^{-1}: 3410—3240, 1746, 1635, and 900; ^{1}H-NMR (C_5D_5N) δ: 0.92 and 1.47 (each 3H, s, 2 Me), 3.40—4.90 (13H), and 7.10 (1H, m, $W_{1/2}=4$ Hz, 14-H). *Anal.* Calcd for $C_{26}H_{40}O_9$: C, 62.88; H, 8.12. Found: C, 62.66; H, 8.15.

14-Deoxy-12-methoxy-andrographolide (**3**): colorless needles, mp 121—124 °C (from MeOH); IR ν_{max}^{KBr} cm^{-1}: 3360, 2940, 1750, 1642, and 923; ^{1}H-NMR ($CDCl_3$) δ: 0.61 and 1.24 (each 3H, s, 2 Me), 3.27 (3H, s, OMe), 3.40 (1H, br t, $J=9$ Hz, 3β-H), 3.48 (1H, ABd, $J=11$ Hz, 19-H_1), 4.83 (4H, m, 15-H_2 and 17-H_2), and 7.31 (1H, m, $W_{1/2}=4$ Hz, 14-H). High-MS *m/z*: 364.225 (M^+). Calcd for $C_{21}H_{32}O_5$: 364.225. The results of precise mass measurements for fragment ions A-D are listed in Table III.

Andrographolide (**8**): colorless plates, mp 218—221 °C (from MeOH), $[\alpha]_D^{25}$ −96.2° ($c=1.00$, C_5H_5N); UV λ_{max}^{MeOH} nm (ε): 223 (13200); IR ν_{max}^{KBr} cm^{-1}: 3340—3200, 1725, 1667, and 909; ^{1}H-NMR [$(CD_3)_2SO$] δ: 0.86 and 1.07 (each 3H, s, 2 Me), 3.18 (1H, m, 3β-H), 3.23 and 3.82 (each 1H, ABd, $J=11$ Hz, 19-H_2), 3.98 (1H, dd, $J=2$ and 10 Hz, 15-H_1), 4.34 (1H, dd, $J=6$ and 10 Hz, 15-H_1), 4.58 and 4.77 (each 1H, s, 17-H_2), 4.85 (1H, br d, $J=5.5$ Hz, 14-H), and 6.49 (1H, t, $J=8$ Hz, 12-H). *Anal.* Calcd for $C_{20}H_{30}O_5$: C, 68.54; H, 8.65. Found: C, 68.49; H, 8.73. This compound was identical with an authentic sample of andrographolide (**8**) on the basis of mixed mp and comparison of IR spectra.

14-Deoxyandrographolide (**4**): colorless needles, mp 172—173 °C (from MeOH), $[\alpha]_D^{23}$ −28.5° ($c=1.04$, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3630, 3500, 1755, 1640, and 898; ^{1}H-NMR ($CDCl_3$) δ: 0.62 and 1.22 (each 3H, s, 2 Me), 3.27 (1H, ABd, $J=11$ Hz, 19-H_1), 3.44 (1H, t, $J=8$ Hz, 3β-H), 4.14 (1H, ABd, $J=11$ Hz, 19-H_1), 4.55 (1H, s, 17-H_1), 4.63 (2H, m, 15-H_2), 4.84 (1H, s, 17-H_1), and 7.08 (1H, m, $W_{1/2}=4$ Hz, 14-H). *Anal.* Calcd for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04. Found: C, 71.84; H, 8.97.

Neoandrographolide (**5**): colorless needles, mp 159—160 °C (from MeOH), $[\alpha]_D^{25.5}$ −34.6° ($c=1.00$, MeOH): UV λ_{max}^{MeOH} nm (ε): end absorption, 220 (7303); IR ν_{max}^{KBr} cm^{-1}: 3380, 1747, 1467, and 910; ^{1}H-NMR (C_5D_5N) δ: 0.65 and 1.20 (each 3H, s, 2 Me), 3.40—5.00 (14H), and 7.12 (1H, m, 14-H). *Anal.* Calcd for $C_{26}H_{40}O_8=1/2H_2O$: C, 63.78; H, 8.44. Found: C, 63.62; H, 8.53.

The structures of 14-deoxyandrographolide (**4**) and neoandrographolide (**5**) were confirmed by chemical reactions and interpretation of physical data of several derivatives.

X-Ray Analysis of Andrographolide (8)——$C_{20}H_{30}O_5$, $M=350.4$. Monoclinic, $a=6.549$ (6), $b=8.000$ (5), $c=17.915$ (16) Å, $\beta=97.24$ (7)°, $Z=2$, $D_x=1.25$ g·cm^{-3}, space group $P2_1$, μ(Mo−$K\alpha$)=1.0 cm^{-1}. Intensity data were obtained for a crystal having dimensions of *ca.* 0.1 × 0.5 × 1.0 mm, mounted on a Syntex R3 four-circle automated diffractometer, equipped with a graphite monochromator, and using a variable rate $\omega/2\theta$ scanning technique within 2θ less than 50°. Three reference reflections monitored periodically showed no significant intensity fluctuations during the course of data collection. A total of 1616 independent reflections was used for the structure analysis. Intensities were corrected for Lorentz and polarization effects, but not for absorption.

Structure Determination and Refinement——The structure was solved by the direct method using the MULTAN program.[7] The resulting E-map revealed the positions of all non-hydrogen atoms. All the hydrogen atoms were found on a difference map during the course of the refinement, which was carried out by a block-diagonal

least-squares method. Thermal parameters were refined anisotropically for all the non-hydrogen atoms and isotropically for the hydrogen atoms. The final R value was 0.057. Atomic coordinates and thermal parameters for non-hydrogen atoms are given in Table IV.

Acetylation of Andrograpanin (1)——Andrograpanin (**1**) (101.4 mg) was acetylated with Ac_2O–C_5H_5N and the reaction product (110 mg) was recrystallized from a mixture of MeOH and H_2O to give andrograpanin acetate (**6**), mp 111—112.5 °C, as colorless needles. IR ν_{max}^{KBr} cm^{-1}: 1740, 1256, and 910; ^{1}H-NMR ($CDCl_3$) δ: 0.68 and 0.96 (each 3H, s, 2 Me), 2.02 (3H, s, OAc), 3.78 and 4.17 (each 1H, ABd, J=11 Hz, 19-H_2), 4.56 (1H, s, 17-H_1), 4.72 (2H, m, 15-H_2), 4.82 (1H, s, 17-H_1), and 7.12 (1H, m, $W_{1/2}$=4 Hz, 14-H); High-MS m/z: 360.231 (M^+). Calcd for $C_{22}H_{34}O_4$: 360.230.

Acid Treatment of Andrograpanin (1)——Andrograpanin (**1**) (38.3 mg) was mixed with EtOH (2 ml) and 1 N HCl aqueous solution (2 ml) and the mixture was heated in a sealed tube at 100 °C for 3.5 h. Excess H_2O was added to the reaction mixture and the whole was extracted with EtOAc. The EtOAc extract, after usual work-up, gave a residue (32 mg), which was purified on a silica gel (1.2 g) column with $CHCl_3$ as the eluent to give isoandrograpanin (**11**) as a syrup. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3640, 1760, and 1650; ^{1}H-NMR ($CDCl_3$) δ: 0.92 and 0.98 (each 3H, s, 2 Me), 1.58 (3H, s, 17-H_3), 3.38 and 3.71 (each 1H, ABd, J=11 Hz, 19-H_2), 4.71 (2H, m, 15-H_2), and 7.10 (1H, m, $W_{1/2}$=4 Hz, 14-H); High-MS m/z: 318.221 (M^+). Calcd for $C_{20}H_{30}O_3$: 318.220.

Acid Hydrolysis of Neoandrographolide (5)——Neoandrographolide (**5**) (214.7 mg) was dissolved in a mixture of EtOH (4 ml) and 1 N HCl aqueous solution (4 ml), and the solution was heated in a sealed tube at 100 °C for 4 h. The reaction mixture was treated as before to give a reaction product (140 mg), which was purified on a silica gel (4.1 g) column with $CHCl_3$–MeOH as the eluent to give the pure isoaglucone (**11**) of neoandrographolide (**5**) as a syrup. High-MS m/z: 318.218 (M^+). Calcd for $C_{20}H_{30}O_3$: 318.220. This substance was identical with isoandrograpanin (**11**) on the basis of comparisons of IR, ^{1}H-NMR, and mass spectra.

Hydrolysis of Neoandrographolide (5) by Takadiastase——A suspension of neoandrographolide (**5**) (300 mg) in H_2O (30 ml) was treated with takadiastase fraction (90 ml) prepared according to the literature,[5] and the mixture was stirred at 32 °C for 118 h, then extracted with EtOAc. The extract was dried and evaporated *in vacuo* to give a residue (164 mg). After purification on a silica gel (4.5 g) column ($CHCl_3$), the product was recrystallized from a mixture of benzene and hexane to give the pure genuine aglucone (**1**) (37 mg), mp 104.5—106.5 °C, $[\alpha]_D^{28}$ −34.3° (c=1.02, $CHCl_3$). High-MS m/z: 318.220 (M^+). Calcd for $C_{20}H_{30}O_3$: 318.220. This substance was identical with natural andrograpanin (**1**) on the basis of mixed mp and comparisons of IR and mass spectra.

Acid Hydrolysis of Andropanoside (2)——Andropanoside (**2**) (190.4 mg) was dissolved in a mixture of EtOH (4 ml) and 1 N HCl aqueous solution and the solution was heated in a sealed tube at 100 °C for 4 h. H_2O (10 ml) was added to the cooled solution and the mixture was extracted with EtOAc (20 ml × 4). The EtOAc extracts were combined, washed with H_2O, dried, and concentrated *in vacuo* to give the amorphous isoaglucone (**12**) (95 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3500, 1760, and 1647; ^{1}H-NMR ($CDCl_3$) δ: 0.91 and 1.24 (each 3H, s, 2 Me), 1.58 (3H, s, 17-H_3), 3.28 (1H, ABd, J=11 Hz, 19-H_1), 3.42 (1H, t, J=8 Hz, 3β-H), 4.18 (1H, ABd, J=11 Hz, 19-H_1), 4.76 (2H, m, 15-H_2), and 7.14 (1H, m, $W_{1/2}$=4 Hz, 14-H). MS m/z: 334 (M^+). This substance was identical with iso-14-deoxyandrographolide (**12**), which is described later, on the basis of comparisons of IR and ^{1}H-NMR spectra. The aqueous layer was concentrated *in vacuo* to a small volume and subjected to paper partition chromatography (Toyo Roshi No. 51; n-BuOH: AcOH : H_2O, 6 : 1 : 2); a spot corresponding to D-glucose (Rf 0.17) was detected.

Acid Treatment of 14-Deoxyandrographolide (4)——14-Deoxyandrographolide (**4**) (129.7 mg) was dissolved in a mixture of EtOH (4 ml) and 1 N HCl aqueous solution (4 ml) and heated in a sealed tube at 100 °C for 4 h. The reaction mixture worked up as before to give a reaction product (127 mg), which was purified on a silica gel (4.4 g) column with $CHCl_3$–Me_2CO as the eluent to give amorphous **12** (94 mg). High-MS m/z: 334.211 (M^+). Calcd for $C_{20}H_{30}O_4$: 334.214. This substance was identical with the isoaglucone (**12**) of andropanoside (**2**) on the basis of comparisons of IR and ^{1}H-NMR spectra.

Hydrolysis of Andropanoside (2) by β-Glucosidase——Andropanoside (**2**) (10.2 mg) was dissolved in an acetate buffer (pH 4.95, 0.1 M) (7 ml) and treated with β-glucosidase (from almonds) (5.9 mg). The mixture was incubated at 36 °C for 50 h with occasional shaking. The mixture was extracted with EtOAc (15 ml × 4). After being washed with H_2O, the extract was dried and evaporated *in vacuo* to give a residue (5 mg) which was purified on a silica gel (0.2 g) column with Et_2O as the eluent to give 14-deoxyandrographolide (**4**) (2 mg), mp 167—170 °C. High-MS m/z: 316.203 ($M^+ - H_2O$). Calcd for $C_{20}H_{28}O_3$: 316.204.

Acetylation of Andropanoside (2)——Andropanoside (**2**) (100.4 mg) was acetylated with Ac_2O–C_5H_5N to give a product which was purified on a silica gel (4.1 g) column ($CHCl_3$–Me_2CO) to give the pentaacetate (**7**) (134 mg) as a syrup. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1758—1740, 1642, and 900; ^{1}H-NMR ($CDCl_3$) δ: 0.74 and 0.97 (each 3H, s, 2 Me), 2.02—2.08 (5 OAc), 3.55 and 3.95 (each 1H, ABd, J=11 Hz, 19-H_2), and 7.12 (1H, m, $W_{1/2}$=4 Hz, 14-H). High-MS m/z: 359.223 (M^+ − OGluc Ac_4). Calcd for $C_{22}H_{31}O_4$: 359.222.

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