Antiviral Agents of Plant Origin. III.[1] Scopadulin, a Novel Tetracyclic Diterpene from *Scoparia dulcis* L.

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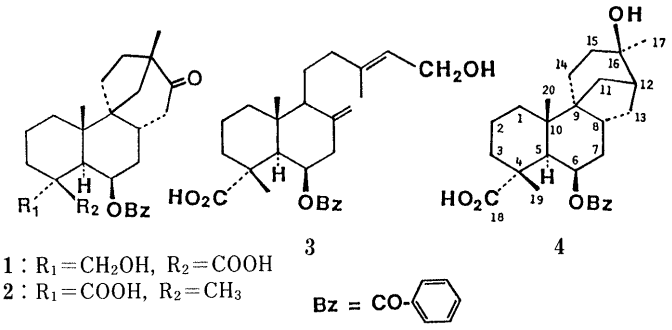
The structure and stereochemistry of scopadulin, a novel aphidicolane-type diterpene isolated from *Scoparia dulcis* L. have been established from spectral data and single-crystal X-ray analysis of its acetone solvate.

Keywords scopadulin; aphidicolane; diterpenoid; *Scoparia dulcis*; Scrophulariaceae; X-ray analysis; antiviral activity

In previous papers,[2] we reported the structures of new diterpenes with an unusual tetracyclic skeleton, scopadulcic acids A (**1**) and B (**2**),[2a,b] as well as labdane-type diterpenoid, scoparic acid A (**3**),[2c] and flavonoids[2d,e] isolated from *Scoparia dulcis* L. (Scrophulariaceae). Among these compounds, scopadulcic acid B (**2**) showed *in vitro* and *in vivo* antiviral activity against herpes simplex virus type 1[3] and also suppressed acid secretion by gastric mucosa stimulated with histamine through inhibition of H^+, K^+-adenosine triphosphatase (ATPase).[4]

Further examination of the chloroform-soluble part of the 70%-ethanolic extract of *Scoparia dulcis* has now resulted in the isolation of a novel tetracyclic diterpene, scopadulin (**4**), which showed mild antiviral activity.[5] This paper deals with the structure determination of scopadulin (**4**).



1 : R_1=CH_2OH, R_2=COOH
2 : R_1=COOH, R_2=CH_3

3 4

Chart 1

Results and Discussion

Scopadulin (**4**), mp 232—234 °C, $[\alpha]_D$ −34.2° (c=0.56 in MeOH) was obtained as colorless prisms (from acetone) in 0.0065% yield and its ultraviolet (UV) and infrared (IR) spectra showed hydroxyl (3200), carbonyl (1715 and 1700 (sh)), and phenyl groups (229, 266, 270 and 276 nm; 1600

TABLE I. Atomic Parameters for Non-hydrogen Atoms

Atom	x	y	z	B_{eq}
C(1)	0.4075 (2)	0.4202 (4)	0.4932 (4)	4.74 (19)
C(2)	0.4049 (2)	0.5062 (5)	0.3829 (5)	5.80 (23)
C(3)	0.4159 (2)	0.4377 (5)	0.2673 (4)	5.42 (21)
C(4)	0.3713 (2)	0.3363 (4)	0.2405 (4)	4.25 (20)
C(5)	0.3678 (2)	0.2576 (4)	0.3572 (3)	3.67 (18)
C(6)	0.3296 (2)	0.1438 (4)	0.3387 (3)	3.99 (18)
C(7)	0.3373 (2)	0.0584 (4)	0.4452 (4)	4.34 (20)
C(8)	0.3266 (2)	0.1247 (4)	0.5636 (3)	3.87 (18)
C(9)	0.3711 (2)	0.2278 (4)	0.5857 (3)	3.83 (18)
C(10)	0.3608 (2)	0.3241 (4)	0.4809 (3)	3.91 (18)
C(11)	0.3561 (2)	0.2626 (5)	0.7164 (4)	4.42 (18)
C(12)	0.3576 (2)	0.1392 (5)	0.7806 (3)	4.35 (20)
C(13)	0.3237 (2)	0.0590 (5)	0.6867 (4)	5.03 (21)
C(14)	0.4350 (2)	0.1841 (4)	0.5946 (3)	3.88 (18)
C(15)	0.4479 (2)	0.0807 (4)	0.6796 (4)	4.53 (20)
C(16)	0.4205 (2)	0.0960 (5)	0.8036 (4)	4.67 (20)
C(17)	0.4245 (3)	−0.0166 (6)	0.8807 (4)	6.26 (30)
C(18)	0.4007 (2)	0.2642 (4)	0.1419 (3)	4.12 (18)
C(19)	0.3133 (2)	0.3869 (5)	0.1873 (4)	5.82 (24)
C(20)	0.3014 (2)	0.3848 (5)	0.4933 (4)	4.87 (21)
C(21)	0.2348 (2)	0.1112 (5)	0.2442 (4)	5.48 (23)
C(22)	0.1710 (2)	0.1356 (5)	0.2535 (5)	5.82 (23)
C(23)	0.1321 (3)	0.0833 (7)	0.1672 (6)	8.05 (32)
C(24)	0.0731 (3)	0.1029 (9)	0.1741 (8)	11.77 (54)
C(25)	0.0522 (2)	0.1715 (7)	0.2657 (8)	11.53 (45)
C(26)	0.0904 (3)	0.2232 (6)	0.3536 (8)	9.49 (39)
C(27)	0.1498 (2)	0.2030 (5)	0.3463 (6)	7.04 (29)
O(1)	0.2671 (1)	0.1742 (3)	0.3261 (2)	4.67 (17)
O(2)	0.4517 (1)	0.1897 (3)	0.8698 (2)	4.94 (18)
O(3)	0.4287 (1)	0.1739 (3)	0.1621 (2)	5.23 (15)
O(4)	0.3965 (2)	0.3128 (3)	0.0335 (2)	5.79 (18)
O(5)	0.2533 (2)	0.0420 (5)	0.1753 (4)	9.00 (24)
C(S1)	0.1694 (3)	0.2795 (8)	0.7963 (7)	9.61 (42)
C(S2)	0.1109 (4)	0.3283 (10)	0.7943 (9)	12.15 (60)
C(S3)	0.2042 (5)	0.2756 (13)	0.9109 (8)	13.76 (66)
O(S1)	0.1889 (3)	0.2520 (8)	0.6986 (7)	14.78 (42)

Estimated standard deviations are given in parentheses. B_{eq} is isotropic equivalent of the anisotropic thermal parameter.

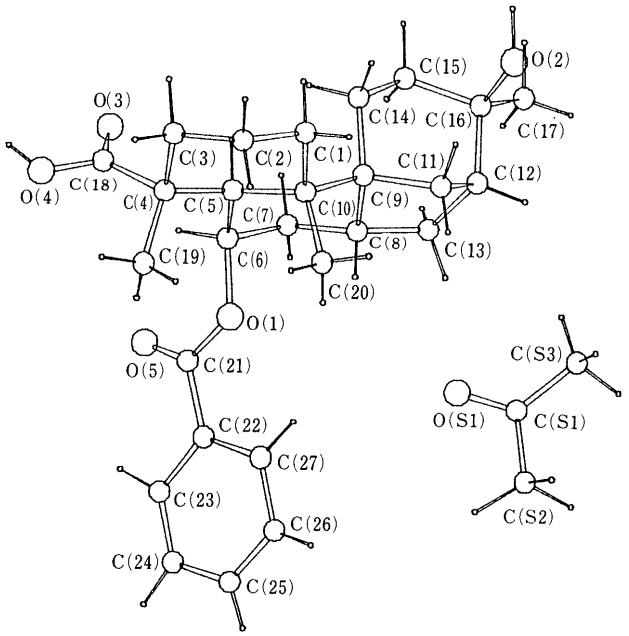
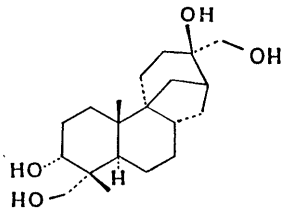


Fig. 1. A Perspective Drawing of the X-Ray Diffraction Structure of Acetone Solvate of Scopadulin (**4**)

TABLE II. Bond Lengths (Å) and Angles (°) with Their e.s.d.'s in Parentheses

Bond	Length (Å)	Bond	Length (Å)
C(1)–C(2)	1.547 (7)	C(1)–C(10)	1.520 (6)
C(2)–C(3)	1.513 (7)	C(3)–C(4)	1.548 (7)
C(4)–C(5)	1.560 (6)	C(4)–C(18)	1.530 (6)
C(4)–C(19)	1.533 (6)	C(5)–C(6)	1.557 (6)
C(5)–C(10)	1.562 (5)	C(6)–C(7)	1.515 (6)
C(6)–O(1)	1.469 (5)	C(7)–C(8)	1.525 (6)
C(8)–C(9)	1.554 (6)	C(8)–C(13)	1.542 (6)
C(9)–C(10)	1.588 (6)	C(9)–C(11)	1.538 (6)
C(9)–C(14)	1.540 (6)	C(10)–C(20)	1.531 (7)
C(11)–C(12)	1.555 (8)	C(12)–C(13)	1.549 (7)
C(12)–C(16)	1.528 (7)	C(14)–C(15)	1.510 (6)
C(15)–C(16)	1.532 (6)	C(16)–C(17)	1.522 (8)
C(16)–O(2)	1.448 (6)	C(18)–O(3)	1.215 (5)
C(18)–O(4)	1.307 (4)	C(21)–C(22)	1.492 (7)
C(21)–O(1)	1.338 (5)	C(21)–O(5)	1.176 (7)
C(22)–C(23)	1.398 (8)	C(22)–C(27)	1.374 (8)
C(23)–C(24)	1.372 (10)	C(24)–C(25)	1.369 (12)
C(25)–C(26)	1.397 (10)	C(26)–C(27)	1.383 (8)
C(S1)–C(S2)	1.445 (12)	C(S1)–C(S3)	1.456 (12)
C(S1)–O(S1)	1.218 (11)		
Bond	**Angle (°)**	**Bond**	**Angle (°)**
C(2)–C(1)–C(10)	111.9 (4)	C(1)–C(2)–C(3)	109.5 (4)
C(2)–C(3)–C(4)	113.6 (4)	C(3)–C(4)–C(5)	108.7 (3)
C(3)–C(4)–C(18)	102.5 (4)	C(3)–C(4)–C(19)	110.5 (4)
C(5)–C(4)–C(18)	108.6 (3)	C(5)–C(4)–C(19)	116.5 (4)
C(18)–C(4)–C(19)	109.2 (4)	C(4)–C(5)–C(6)	114.1 (3)
C(4)–C(5)–C(10)	116.8 (4)	C(6)–C(5)–C(10)	115.3 (3)
C(5)–C(6)–C(7)	112.1 (3)	C(5)–C(6)–O(1)	111.0 (4)
C(7)–C(6)–O(1)	107.3 (3)	C(6)–C(7)–C(8)	109.1 (4)
C(7)–C(8)–C(9)	111.6 (3)	C(7)–C(8)–C(13)	121.7 (4)
C(9)–C(8)–C(13)	106.0 (3)	C(8)–C(9)–C(10)	108.8 (3)
C(8)–C(9)–C(11)	99.3 (3)	C(8)–C(9)–C(14)	112.6 (4)
C(10)–C(9)–C(11)	117.9 (4)	C(10)–C(9)–C(14)	111.8 (3)
C(11)–C(9)–C(14)	105.9 (3)	C(1)–C(10)–C(5)	108.5 (3)
C(1)–C(10)–C(9)	109.9 (3)	C(1)–C(10)–C(20)	107.3 (4)
C(5)–C(10)–C(9)	106.5 (3)	C(5)–C(10)–C(20)	114.6 (3)
C(9)–C(10)–C(20)	110.0 (3)	C(9)–C(11)–C(12)	101.1 (4)
C(11)–C(12)–C(13)	102.6 (3)	C(11)–C(12)–C(16)	111.1 (4)
C(13)–C(12)–C(16)	111.3 (4)	C(8)–C(13)–C(12)	105.2 (4)
C(9)–C(14)–C(15)	116.3 (4)	C(14)–C(15)–C(16)	112.6 (4)
C(12)–C(16)–C(15)	108.0 (3)	C(12)–C(16)–C(17)	112.8 (4)
C(12)–C(16)–O(2)	106.8 (4)	C(15)–C(16)–C(17)	112.5 (4)
C(15)–C(16)–O(2)	108.3 (4)	C(17)–C(16)–O(2)	108.1 (4)
C(4)–C(18)–O(3)	124.0 (3)	C(4)–C(18)–O(4)	113.8 (4)
O(3)–C(18)–O(4)	122.1 (4)	C(22)–C(21)–O(1)	111.6 (4)
C(22)–C(21)–O(5)	123.1 (5)	O(1)–C(21)–O(5)	125.2 (4)
C(21)–C(22)–C(23)	118.0 (5)	C(21)–C(22)–C(27)	122.1 (4)
C(23)–C(22)–C(27)	119.8 (5)	C(22)–C(23)–C(24)	119.5 (7)
C(23)–C(24)–C(25)	120.5 (7)	C(24)–C(25)–C(26)	120.8 (5)
C(25)–C(26)–C(27)	118.4 (7)	C(22)–C(27)–C(26)	120.9 (6)
C(6)–O(1)–C(21)	116.4 (3)	C(S2)–C(S1)–C(S3)	119.6 (8)
C(S2)–C(S1)–O(S1)	117.3 (7)	C(S3)–C(S1)–O(S1)	122.9 (8)

and 1580 cm^{-1}). The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of **4** indicated the presence of a carbonyl (δ_C 181.4), a benzoyl (δ_H 7.53 (3H, m) and 8.33 (2H, d, J=7.8 Hz); δ_C 129.0 (d), 130.0 (2d), 131.5 (s), 133.3 (2d) and 166.1 (s)), an oxygenated methine (δ_H 6.01; δ_C 75.2 (d)), and three tertiary methyl groups (δ_H 1.29, 1.59 and 1.73). The electron-impact mass spectrum (EI-MS) showed the fragment ions at m/z 425 (M^+-Me), 422 (M^+-H_2O), 300 ($M^+-H_2O-C_6H_5COOH$), 105 ($C_6H_5CO^+$) and 77 ($C_6H_5^+$). These spectral data are similar to those of scopadulcic acid B (**2**).[2a]



aphidicolin

5

Chart 2

However, the ^{13}C-NMR spectrum of **4** showed a signal due to an oxygenated quarternary sp^3 carbon at δ 71.3 and no signal of ketonic carbon. The molecular formula of **4** was suggested to be $C_{27}H_{36}O_5$ on the basis of NMR and MS data. Finally, the relative structure of **4** was determined by X-ray crystallography on a single crystal of **4** obtained by crystallization from acetone. The crystal structure was solved by the direct method using 1992 independent reflection data. The atomic parameters of non-hydrogen atoms obtained were refined by the full matrix least-squares method to R=0.071, and then to R=0.052 including those of hydrogen atoms located from a ΔF map. The resultant atomic parameters for the non-hydrogen atoms and the bond lengths and angles are listed in Tables I and II, respectively, and the computer drawing of the structure of **4** is given as a solvate with one equivalent mole of acetone as shown in Fig. 1. The elemental analysis for **4** is fully explainable for this result.

As presented above, the carbon skeleton of scopadulin (**4**) was found to be the same as aphidicolin (**5**) isolated from a fungus *Cephalosporium aphidicola* PETH.[6] This is the first example of an aphidicolin type diterpenoid from a higher plant.[7] It is very interesting that three different types of diterpenoids, scopadulcic acid B (**2**), scoparic acid A (**3**) and scopadulin (**4**), were isolated from *Scoparia dulcis*. Ackland and Handon proposed that aphidicolin would be biosynthesized from labdane vir pimarane.[8] Therefore, we suppose that **4** is also derived from **3** vir pimarane and then transformed to **2**.

Experimental

Apparatus Melting point was determined on a Yanagimoto micro-melting point apparatus and is uncorrected. UV spectrum was taken on a Hitachi 220S spectrophotometer and IR spectrum was recorded on a Hitachi 260-10 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL GX-270 (270 MHz) and Varian XL-200 (50.3 MHz) spectrophotometer, respectively. Chemical shifts are given in δ-values (ppm) with tetramethylsilane as an internal standard. Mass spectra were measured with a JEOL JMS-D 200 mass spectrometer at an ionization voltage of 70 eV. Optical rotation was recorded on a JASCO DIP-140 digital polarimeter.

Isolation of Scopadulin (4) The $CHCl_3$-soluble part (64 g) of the 70%-ethanolic extract from *Scoparia dulcis* was purified by repeated silica gel column chromatography ($CHCl_3$–MeOH as eluants) to furnish a fraction containing crude scopadulin. Further purification of this fraction obtained from 10% MeOH in $CHCl_3$ eluate (1.8 g) by medium-pressure liquid chromatography (MERCK Silica gel 60, 2.5×23 cm, $CHCl_3$) afforded **4** (98 mg). Scopadulin (**4**): UV λ_{max}^{MeOH} nm (log ε): 229 (4.37), 266 (2.82), 270 (2.84), 276 (2.80). ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 1.06 (1H, dd, J=13.2, 7.8 Hz), 1.29 (3H, s), 1.59 (3H, s), 1.73 (3H, s), 2.42 (1H, d, J=10.7 Hz), 2.63 (1H, m), 3.02 (1H, d, J=2.4 Hz), 6.01 (1H, br s), 7.53 (3H, m), 8.33 (2H, d, J=7.8 Hz). ^{13}C-NMR (50.3 MHz, pyridine-d_5) δ: 18.7 (q), 19.0 (t), 20.2 (q), 25.6 (t), 28.5 (q), 31.5 (t), 33.1 (t), 33.4 (d), 33.8 (t), 35.2 (d), 36.0 (t), 40.5 (s), 41.0 (t), 44.4 (d), 47.5 (t), 48.0 (s), 48.5 (s), 71.3 (s),

75.2 (d), 129.0 (d), 130.0 (2d), 131.5 (s), 133.3 (2d), 166.1 (s), 181.4 (s). *Anal.* Calcd for $C_{27}H_{36}O_5 \cdot C_3H_6O$: C, 72.25; H, 8.49. Found: C, 72.60; H, 8.42. MS *m/z*: 425 (1, $M^+ - Me$), 422 (1, $M^+ - H_2O$), 300 (25, $M^+ - H_2O - C_6H_5COOH$), 105 (100, $C_6H_5CO^+$), 77 (40, $C_6H_5^+$). High-resolution MS: Found 425.231, Calcd for $C_{26}H_{33}O_5$ 425.233 ($M^+ - 15$); Found 422.248, Calcd for $C_{27}H_{34}O_4$ 422.246 ($M^+ - 18$); Found 300.213, Calcd for $C_{20}H_{28}O_2$ 300.209 ($M^+ - 18 - 122$).

X-Ray Crystallographic Analysis of Scopadulin (4) as Its Acetone Solvate A crystal of dimensions $0.2 \times 0.3 \times 0.3$ mm was selected for X-ray measurements. Crystal Data: $C_{27}H_{36}O_5 \cdot C_3H_6O$, monoclinic, space group C2, $Z=4$, $a=22.859(9)$, $b=11.241(2)$, $c=10.956(1)$ Å, $U=2812.3$ Å^3, $\beta=92.63(2)$, $D_c=1.178$ g/cm^3, $\mu(CuK_\alpha)=6.13$ cm^{-1}, $F(000)=1080$. Reflection data [$|F_o| > 3\sigma(|F_o|)$, $\theta \leqq 60°$] were collected on a Rigaku AFC-5RU diffractometer with an $\omega-2\theta$ scan mode using graphite monochromated CuK_α radiation ($\lambda=1.54178$ Å). The structure was solved by direct methods using MULTAN and was refined by the full-matrix least-squares method on a FACOM M380 computer in the Data Processing Center of Kyoto University, using KPPKRAY Programs. Atomic coordinates, bond lengths and bond angles are given in Tables I and II.

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