Biologically Active Constituents of *Arnebia euchroma*: Structure of Arnebinol, an Ansa-Type Monoterpenylbenzenoid with Inhibitory Activity on Prostaglandin Biosynthesis

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Three phenolic compounds were isolated from the roots of *Arnebia euchroma* as inhibitors of *in vitro* prostaglandin biosynthesis. Two known compounds were identified as shikonofurans and des-*O*-methyllasioldiplodin. The other new compound was named arnebinol and its structure was elucidated as a novel ansa-type monoterpenylbenzenoid derivative.

Keywords *Arnebia euchroma*; Boraginaceae; arnebinol; shikonofuran; des-*O*-methyllasioldiplodin; inhibitor; prostaglandin

In the course of studies to clarify bioactive principles of medicinal plants used in traditional medicines,¹⁾ we have identified a number of natural products possessing inhibitory effect on an *in vitro* prostaglandin (PG) biosynthesizing enzyme system (PG synthetase).^{1c-e)} Gingerols and diarylheptanoids have been isolated as the inhibitors of PG synthetase, respectively from the rhizomes of *Zingiber officinale* and *Alpinia officinarum* which have been used as medicinal drugs in the traditional Japanese Kampo medicine.^{1c,d)} The efficacy of such an inhibitor of PG biosynthesis was proved in a pre-clinical test by Hirai and Tamura on paeony root bark.²⁾ Administration of an aqueous extract of its root bark to humans resulted in a lowering of platelet aggregation in parallel with a decrease of thromboxane A₂ (TXA₂) synthesis in platelets. The inhibitory effect of the extract on arachidonate cyclooxygenase was seen in a decrease of the level of HHT (12-hydroxy-5,8,10-heptadecatrienoic acid), while the formation of 15-HETE (15-hydroxyeicosatetraenoic acid), a product of lipoxygenase, was not affected. Paeonol, the main constituent of paeony root bark inhibited *in vitro* PG biosynthesis at a level comparable to aspirin.^{3,4)} The results indicate that paeonol inhibited platelet cyclooxygenase to decrease platelet aggregation induced by arachidonic acid.²⁾ In our subsequent studies on the inhibitors of PG biosynthesis, cinnamoylphenols, isoflavonoids and a benzoic acid derivative were isolated from *Dalbergia odorifera*,³⁾ cinnamoyl- β -phenethylamines from *Mucuna birdiwoodiana*,⁵⁾ and a dihydrostyrene from *Allium chinensis*.⁶⁾ Some of them also inhibited *in vitro* platelet aggregation induced by arachidonic acid.^{3,5,6)}

The roots of *Arnebia euchroma* (ROYLE) JOHNST (Japanese name nanshikon) have been used as a medicinal drug for antipyretic and antibacterial purposes in traditional oriental medicines.⁷⁾ In previous communications we briefly reported on the identification and structural elucidation of several constituents possessing inhibitory activity to PG synthetase from this material.⁸⁾ This paper describes in detail the isolation, identification and structural elucidation of three phenolic compounds, shikonofuran B and C (1), arnebinol (2) and des-*O*-methyllasioldiplodin (4).

A hot aqueous extract of *A. euchroma* roots inhibited PG synthetase reaction by 35% at a concentration of 750 μ g/ml. A preliminary fractionation of the aqueous extract with XAD-2 followed by inhibition testing of the fractions revealed that bioactive compounds contained in the extract

were not naphthoquinone pigments such as alkannin (5), the main constituents of this medicinal plant, since the most active fraction eluted from the XAD-2 column with methanol contained no naphthoquinones (Chart 1). In spite

Chart 1. Extraction of *A. euchroma* Roots and Fractionation of Extract
a) Inhibition % for *in vitro* PG biosynthesis.

Chart 2. Chromatographic Separation of Active Fractions
a) Inhibition % for *in vitro* PG biosynthesis.

Chart 3. Chromatographic Separation of Active Fractions

a) Inhibition % for *in vitro* PG biosynthesis. b) IC_{50} values for *in vitro* PG biosynthesis.

of extensive fractionation and separation on a hot aqueous extract from 5 kg of roots of *A. euchroma*, no fruitful results have so far been obtained in isolating active compounds. A small scale extraction with organic solvents followed by inhibition testing revealed that the active compounds were effectively extracted by organic solvents and that chloroform extract exhibited the strongest inhibition to PG biosynthesis.

Based on these observations 5 kg of *A. euchroma* roots was extracted with hexane to remove naphthoquinones and then extracted with methanol. The methanol extracts were fractionated with hexane and methanol to remove heavily coloured pigments. The chloroform fraction (fr. I-1) that was obtained upon further fractionation of the methanol layer with chloroform and water was highly active as shown in Chart 1. In the following separation and fractionation process each fraction was monitored by testing its inhibitory activity against PG synthetase. For example, silica-gel column chromatography gave two fractions (frs. C and E) of relatively high activity (Chart 2). The extensive separation of the fr. C by repeated column chromatographies with silica-gel, Lobar RP-8 and Sephadex LH-20 finally afforded four compounds, tentatively designated as NS-3, NS-4, NS-6 and NS-7 (Chart 3). The fr. E also afforded two compounds, NS-1 and NS-2, of which NS-1 could not be characterized due to its small yield (Chart 2). It is clear from that the isolated compounds cannot represent all the activity present in the fr. I-1. Many other compounds contained in the chloroform soluble fraction (fr. I-1) must contribute to the overall activity.

By chemical and spectral investigations NS-2 was identified as a mixture of shikonofuran B and C (**1**), which had been isolated from the roots of *Lithospermum eryth-*

rorhizon.⁹⁾ Gas chromatography-mass spectra (GC-MS) analysis of the methyl esters of fatty acids obtained by methanolysis of shikonofurans (**1**) clarified that the ester group consists of α -methylbutyrate and isovalerate in a ratio of 1.4 : 1. Thus, NS-2 is a mixture of shikonofurans B and C (**1**) in a ratio of 1.4 : 1.

A colourless compound NS-4 which was named arnebinol (**2**) was obtained in 0.0004 % yield and its IC_{50} value for PG synthetase was 29.5 μ M. High resolution mass spectra (HR MS) gave an element composition of $C_{16}H_{20}O_2$. Its spectral data indicated NS-4 to be a new compound. The proton nuclear magnetic resonance (1H -NMR) spectrum that was measured at room temperature gave signals corresponding to the protons of 1,2,4-substituted benzene, two methyls on olefinic carbons, two olefinic methines and four aliphatic methylenes. Of the four aliphatic methylenes only one gave a broad but clear triplet (δ 2.34, J = 7 Hz), while the others gave two pairs of extremely broad singlets (δ 2.14, 2.49 and δ 3.07, 3.30) and a broad singlet (δ 4.52) (Fig. 1A and Table I). The broad signals of the methylenes indicate that a steric hindrance preventing the free rotation of the three methylenes causes broadening of the signals by an equilibrium between different conformations. In the NMR spectrum taken at a low temperature (239 K), the three methylenes appear as sharp signals, which prove the conformational equilibrium of arnebinol (**2**) (Fig. 1A). Proton homonuclear decoupling and proton correlation spectroscopy (1H -COSY) data clarified two C_5 partial structures of isopentenyl skeleton, which were combined into a C_{10} partial structure with geranyl skeleton according to biogenetical considerations. Each olefinic methine proton couples with adjacent methylene

Fig. 1. ^1H -NMR Spectra of Arnebinol (A, **2**) and Isoarnebinol (B, **3**) at Varied Temperatures

TABLE I. NMR Assignment of Arnebinol (**2**)

Atom No.	^1H -NMR (δ)	^{13}C -NMR (δ) ^{a)}
2	4.52 (2H, brs)	— ^{b)}
3	—	140.2
4	5.51 (1H, br t, $J=7$ Hz)	127.0
5	2.14, 2.49 (1H $\times$ 2, br s $\times$ 2)	25.3 ^{c)}
6	2.34 (2H, br t, $J=7$ Hz)	25.8 ^{c)}
7	—	133.6
8	5.67 (1H, br t, $J=7$ Hz)	124.2
9	3.07, 3.30 (1H $\times$ 2, br s $\times$ 2)	39.3
10	—	146.8
11	6.59 (1H, d, $J=8.5$ Hz)	118.2
12	6.55 (1H, dd, $J=3, 8.5$ Hz)	115.1
13	—	151.8
14	7.44 (1H, d, $J=3$ Hz)	115.2
15	—	132.6
16	1.50 (3H, s)	14.8
17	1.24 (3H, s)	12.6

a) ^{13}C -NMR signals were assigned based on CH-COSY. b) Overlapped with solvent signal. c) Signals had no correlation in CH-COSY and assignments may be interchangeable.

Fig. 2. Drawing of Arnebinol (**2**) with Coordinates of X-Ray Analysis

protons as well as with allylic methyl protons with significant coupling constants. The methylene protons at δ 2.34 (C-6) coupled with the allylic methine proton at δ 5.67 (C-8) with a very small coupling constant causing broadening in the signals. This allows complete assignment of ^1H -NMR signals of the monoterpene moiety. The NMR data and biogenetical considerations indicated that the configuration of the two double bonds should be *E*.

Geranylhydroquinone (**6**) and alliodorol (**7**), monoterpenylhydroquinones, have been isolated from Boraginaceae plants and are postulated to be the precursors of other phenolic and quinonic compounds occurring in these plants.¹⁰⁾ This suggested that arnebinol (**2**) is a dehydro-derivative of alliodorol (**7**) which forms an ether linkage with one of the oxygen atoms of the hydroquinone. There were two possible structures (**2,3**) for arnebinol. However,

the presence of steric hindrance that prevented free rotation of the methylenes indicated that the ansa-type structure (**2**) with a 12-membered ether ring was the more probable. The structure of arnebinol (**2**) was unambiguously established by X-ray analysis. Arnebinol (**2**) gave a crystal suitable for X-ray analysis from benzene with a space group of $P2_1$. The structure was solved by the direct method and refined by least-squares. The final *R* value was 0.052 with anisotropic temperature factors for carbon and oxygen atoms, and isotropic temperature factors for hydrogen atoms. A drawing of the structure from X-ray analysis is shown in Fig. 2.

The biogenesis of shikonofuran (**1**) and arnebinol (**2**) is a very interesting subject. Shikonin, alkannin (**5**) and shikonofuran (**1**) have been suggested to be derived from geranylhydroquinone (**6**) via a hydroxylated geranylhydroquinone (**8**), though further modification is required to form a furan and naphthoquinone group.¹¹⁾ The

Chart 4. Biogenesis of Shikonofurane (1), Arnebinol (2) and Alkannin (5)

biogenesis of arnebinol (2) is readily accounted for by the dehydration reaction of alliodorol (7) which has a hydroxy group at pro-*E* methyl of geranylhydroquinone. Mori *et al.* attempted to synthesize arnebinol (2) according to the biogenetic scheme (Chart 4) and obtained arnebinol (2) and unnatural isoarnebinol (3) by the cyclization of a derivative of alliodorol.¹²⁾ The dynamic NMR spectra of arnebinol (2) and isoarnebinol (3) that were measured at different temperatures clearly demonstrate arnebinol (2) to be the more hindered compound. The methylene groups of isoarnebinol (3) rotate freely above 28 °C (301 K), while the rotation of the methylene groups of arnebinol (2) is hindered up to 44 °C (317 K) (Fig. 1). At a low temperature (239 K) arnebinol (2) gave a spectrum of one conformation in a solution.

NS-7 was obtained as colourless crystals in yield of 0.0004% and its IC₅₀ value for the inhibition of *in vitro* PG biosynthesis was 71.5 μM. Molecular formula was given by HR MS as C₁₆H₂₂O₄. The ¹³C-NMR spectrum gave signals arising from seven methylene groups, indicating NS-7 is not a compound deriving from geranylhydroquinone (6) as are shikonofuran (1) and arnebinol (2). The ¹H-NMR spectrum gave signals assignable to two aromatic protons at *meta* position in a benzene ring, a secondary methyl appearing as a doublet and a methine showing ddq coupling pattern, suggesting NS-7 is a polyketide macrolide. It was finally identified by direct comparison of samples as des-*O*-methylasiadiplodin (4) which had been isolated from the fungus *Lasiodiplodia theobromae*.¹³⁾ Lasiodiplodin, a methyl ether of des-*O*-methylasiadiplodin, was isolated from the plant, *Euphorbia apendens* (Euphorbiaceae), as a potent antileukemic substance.¹⁴⁾ However, the polyketides of macrolide structure are typical of fungal metabolites and not often isolated from plants. Des-*O*-methylasiadiplodin (4) may derive from a fungus grown on the plant by symbiosis or just contamination.

The inhibitory activities of shikonofurans (1), arnebinol (2) and des-*O*-methylasiadiplodin (4) support our view on the inhibitory activity of phenolic compounds to *in vitro* PG biosynthesis. They belong to the rather more potent phenolic inhibitors so far observed in our studies.^{1c-e, 3-5)} The presence of free phenolic and lipophilic groups is their common structural feature as in gingerols, diarylheptanoids, cinnamoyl-phenols and isoflavonoids. As far as our studies on the inhibitors of PG biosynthesis from plant origin are concerned, α-cyperone, a sesquiterpene, obtained from

Cyperus rotundus is the sole exception.^{1e)}

Experimental

NMR spectra were measured on a JEOL FX-100 or FX-400 spectrometer, MS on a JEOL JMS-DX300, infrared (IR) on a JASCO model 701 G and ultraviolet (UV) on a Hitachi spectrometer model 100-60. GC-MS was performed on a Shimadzu LKB GC-MS 9000 combined with a GC-MS PAC 300 computer system and Chromatopac 4B Integrator. Melting points were determined on a Yanagimoto micro melting point apparatus and are not corrected.

Assay of PG Biosynthesis Bioassay of PG biosynthesis was carried out with radioactive arachidonic acid and a microsomal enzyme preparation from rabbit kidney medulla as described in a previous paper.^{1e)}

Preliminary Fractionation of a Hot Aqueous Extract The roots of *Arnebia euchroma* (nanshikon) purchased from Uchida Wakanyaku Co. (Tokyo) were extracted with 15 ml water per g of material at 90 °C for 6 h. A solution of the extract obtained upon filtration while still hot was concentrated *in vacuo*, frozen and dried to give the hot aqueous extract, which showed 35% inhibition against the PG biosynthesizing enzyme system at a concentration of 750 μg/ml. The extract (4 g) was dissolved in water (40 ml), applied on a XAD-2 column (110 g; 26 mm × 350 mm) and eluted with water at a flow rate of 2 ml/min. The column was successively eluted with 50% MeOH (1350 ml), MeOH (850 ml) and acetone (1050 ml), H₂O and 50% MeOH eluents showed no significant activities, while the MeOH and acetone fractions inhibited PG biosynthesis by 97 and 75.6%, respectively, at a concentration of 750 μg/ml.

Preliminary Extraction Experiment with Organic Solvents The roots of *A. euchroma* (50 g) were successively extracted twice with 500 ml each of hexane, CHCl₃, MeOH and water. Extracts were evaporated to dryness *in vacuo* and their inhibitory activities tested. The dried CHCl₃ extract was obtained in 0.7% yield and inhibited PG synthetase by 73.5% at a concentration of 200 μg/ml.

Extraction and Fractionation The roots of *A. euchroma* (5 kg) were extracted with boiling hexane (30 l × 6 times) to remove naphthoquinone pigments and then extracted with MeOH (30 l × 4 times) at 60 °C. A small portion of the MeOH extract was evaporated to dryness and tested for inhibition of PG biosynthesis. The dried extract inhibited PG biosynthesis 73.4% at a concentration of 100 μg/ml. The MeOH solution was concentrated to 4 l *in vacuo* and fractionated with hexane (6 l) after adding a small volume of water. This process was repeated 6 times to give an upper hexane layer, a middle layer containing insoluble material and a lower MeOH layer. The MeOH layer was concentrated to 1 l *in vacuo* and fractionated with CHCl₃ and water (2:1, 3 l) three times. The CHCl₃ layer was evaporated to dryness to give an extract (55 g), which showed 82.7% inhibition (100 μg/ml) against the PG biosynthesizing enzyme system. The dried CHCl₃ extract (15 g) was subjected to chromatography over a silica-gel (500 g) and successively eluted with benzene, benzene-acetone (49:1, 19:1, 1:1, v/v) and MeOH. The two fractions eluted with benzene-acetone (49:1) exhibited the strongest inhibitory effect, 77.8% and 70.9% inhibition at 50 μg/mg, respectively, and were designated as fr. C (360 mg) and fr. E (740 mg).

Isolation of Shikonofuran (NS-2:1) The fr. E (240 mg) was further fractionated with a silica gel column (24 g) using CHCl₃, CHCl₃-MeOH and MeOH as eluents. The most active fr. E7 (85 mg) was obtained by CHCl₃ elution and inhibited PG biosynthesis by 79.2% at a 50 μg/ml concentration. The fr. E7 was further separated with a silica-gel column

TABLE II. Fractional Coordinates, $B_{eq}(\text{\AA}^2)$, $U_{eq}(\text{\AA}^2)$ and $U(ij)(\text{\AA}^2)$ for Non Hydrogen Atoms with Estimated Standard Deviations in Parentheses ($\times 10^4$)

Atom	x	y	z	B_{eq}	U_{eq}	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	-1094 (4)	-134 (0)	2009 (5)	48400 (400)	613 (5)	812 (16)	279 (12)	797 (19)	89 (12)	517 (15)	9 (13)
C(2)	-2230 (5)	-517 (3)	3592 (7)	42900 (600)	543 (7)	696 (24)	277 (16)	689 (25)	108 (16)	359 (21)	99 (17)
C(3)	-4065 (5)	-126 (3)	3394 (6)	35900 (500)	454 (6)	624 (19)	259 (15)	499 (18)	2 (15)	213 (15)	6 (14)
C(4)	-4782 (5)	234 (3)	5109 (7)	35700 (400)	453 (6)	571 (18)	287 (16)	521 (17)	4 (13)	234 (14)	19 (14)
C(5)	-6552 (5)	700 (3)	5240 (8)	46300 (600)	587 (8)	524 (18)	370 (18)	894 (30)	-53 (16)	310 (19)	-96 (20)
C(6)	-6387 (5)	1715 (3)	6220 (7)	41600 (600)	527 (7)	525 (20)	380 (20)	707 (25)	-7 (15)	342 (18)	-68 (18)
C(7)	-5103 (5)	2335 (3)	5100 (6)	33100 (400)	420 (5)	480 (14)	305 (16)	493 (17)	45 (12)	201 (13)	-29 (13)
C(8)	-3585 (4)	2607 (3)	6048 (5)	33500 (400)	425 (5)	542 (17)	314 (15)	436 (15)	12 (14)	196 (13)	-64 (15)
C(9)	-2143 (5)	3115 (3)	4954 (7)	39400 (500)	498 (6)	566 (19)	309 (15)	645 (21)	-77 (16)	272 (17)	-84 (15)
C(10)	-506 (4)	2851 (3)	1575 (5)	34000 (400)	431 (6)	404 (18)	370 (16)	531 (19)	-12 (13)	153 (14)	52 (15)
C(11)	90 (6)	2235 (3)	29 (7)	39600 (500)	502 (6)	519 (17)	448 (19)	564 (19)	18 (16)	260 (15)	70 (16)
C(12)	-109 (5)	1240 (3)	183 (6)	39000 (500)	494 (6)	533 (18)	408 (19)	566 (20)	72 (16)	270 (17)	1 (16)
C(13)	-865 (5)	857 (3)	1967 (6)	36200 (500)	458 (6)	503 (17)	287 (16)	608 (20)	73 (14)	246 (15)	17 (14)
C(14)	-1367 (4)	1470 (3)	3556 (6)	34100 (400)	432 (6)	451 (15)	337 (16)	526 (19)	43 (13)	213 (14)	29 (14)
C(15)	-1283 (4)	2469 (3)	3357 (5)	31400 (400)	398 (5)	393 (13)	338 (16)	475 (16)	-27 (12)	136 (12)	-9 (13)
C(16)	-4953 (8)	-200 (4)	1238 (7)	58100 (800)	736 (0)	985 (32)	698 (32)	527 (22)	-51 (30)	58 (22)	-179 (24)
C(17)	-5601 (6)	2588 (4)	2819 (7)	49700 (600)	629 (8)	676 (24)	613 (26)	598 (21)	23 (24)	30 (19)	78 (23)
O(10)	-385 (4)	3834 (2)	1440 (5)	46800 (400)	592 (5)	791 (18)	336 (13)	680 (17)	-65 (14)	323 (14)	44 (13)

$$T = \exp\{-2\pi^2(a^*U_{11}h^2 + b^*U_{22}k^2 + c^*U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl)\}.$$

$$U_{eq} = \frac{1}{3} \sum_i \sum_j U_{ij} a_i^* a_j^* a_i a_j.$$

(20 g) and two active fractions (frs. E71 and E72) were eluted with benzene. Fraction, E71 was finally separated with a Lobar (RP-8) column by eluting with MeOH-H₂O (8.5:1.5) to give NS-1 (1.4 mg), which inhibited PG biosynthesis 31.8% (20 μ g/ml). Fraction E72 was further chromatographed on LH-20 to give an active compound designated as NS-2 (1: 42 mg). The final yield of NS-2 was 0.003% and its inhibition to PG biosynthesis at a concentration of 20 μ g/ml was 72.5%.

Isolation of NS-3 (Arnebifuranone)^(8c) and NS-4 (Arnebinol: 2) The fr. C (360 mg) obtained in the first silica-gel column chromatography was further fractionated on a silica-gel (36 g) column. Fractions C6 and C7 which were eluted with hexane-benzene (1:2) and benzene gave a mixture of several of several compounds (180 mg) of relatively high activity (66% inhibition at a concentration of 20 μ g/ml). The mixture (180 mg) was further separated by a Lobar RP-8 column with 85% MeOH to give fr. C67B (49 mg) and fr. C67D (73 mg), which inhibited PG biosynthesis 68.1% and 35.7% (20 μ g/ml), respectively. Fraction C67B was separated into two active fractions with a silica-gel (3 g) column using benzene as a solvent. One of the two fractions; fr. C67B2, was first washed with hexane and recrystallized from 20% MeOH to give colourless crystals of NS-4 (5.4 mg), which inhibited PG biosynthesis 75.0% at a concentration of 20 μ g/ml. The other fraction, fr. C67B4, was chromatographed on a Sephadex LH-20 (15 $\times$ 1.7 cm) and NS-3 (14 mg) was obtained as an orange oil by collecting a yellow band eluted with MeOH; it inhibited PG biosynthesis 18.7% at a concentration of 20 μ g/ml.

Isolation of NS-6 (Arnebinone)^(8b) and NS-7 (Des-O-methylasiodiplodin: 4) The fr. C67D (73 mg) described above was further separated with a Sephadex LH-20 column (7 $\times$ 1.6 cm) to give two active fractions, fr. C67D1 and fr. C67D2. NS-6 (60 mg) was obtained as an orange oil from the former fraction and the latter gave NS-7 (5 mg) as colourless crystals from MeOH. It inhibited PG biosynthesis 62.2% at a concentration of 20 μ g/ml.

Shikonofuran B and C (NS-2: 1) A slightly yellow oil. HR MS: Calcd for C₂₁H₂₆O₅ 358.1779, Found 358.1760. MS m/z (rel. int.): 358 (M⁺, 2.4), 256 (37.4), 85 (63.7), 57 (100). ¹H-NMR (100 MHz, CDCl₃) δ : 0.88 (3H, t, J =7 Hz), 1.16 (3H, d, J =7 Hz), 1.63 (3H, s), 1.70 (3H, s), 2.44 (1H, m), 2.57 (2H, t, J =7 Hz), 5.08 (1H, brt, J =7 Hz), 5.78 (1H, t, J =7 Hz), 6.43 (1H, brs), 6.64 (1H, dd, J =2.5, 9 Hz), 6.70 (1H, brs), 6.83 (1H, d, J =9 Hz), 7.00 (1H, d, J =2.5 Hz), 7.43 (1H, brs). Inhibition of PG biosynthesis: 72.5% (20 μ g/ml).

Identification of Fatty Acids of the Ester Group in Shikonofurans (1) A sample of shikonofuran (1 mg) in 3% HCl/MeOH was sealed in a glass tube and heated at 100 °C for 3 h. Water (100–200 μ l) and hexane (300 μ l) were added to the cooled reaction mixture. After vortexing the hexane layer was subjected to GC-MS analysis. Column: DEGS (GL Science) 0.28 mm $\times$ 1.5 m, carrier gas: N₂ (flow rate 20 ml/min), column temp.: 50 °C, inj. temp: 120 °C, retention times: methyl α -methylbutyrate 5.36 min; methyl isovalerate 5.59 min. Peak ratio of methyl α -methylbutyrate and methyl isovalerate was 1.4:1.

TABLE III. Fractional Coordinates ($\times 10^3$), $B_{eq}(\text{\AA}^2)$ and $U_{eq}(\text{\AA}^2 \times 10^2)$ for Hydrogen Atoms with Estimated Standard Deviations in Parentheses

Atom	x	y	z	B_{eq}	U_{eq}
H(C2)	-159 (4)	-36 (4)	520 (6)	4.0 (1)	5.3 (1.1)
H'(C2)	-219 (5)	-116 (4)	337 (7)	5.0 (1)	5.9 (1.3)
H(C4)	-401 (6)	19 (4)	661 (7)	4.0 (1)	5.4 (1.1)
H(C5)	-729 (8)	41 (5)	606 (11)	8.0 (0)	9.9 (0.0)
H'(C5)	-716 (5)	75 (4)	359 (7)	5.0 (1)	6.7 (1.4)
H(C6)	-774 (7)	205 (5)	595 (10)	7.0 (0)	9.5 (0.0)
H'(C6)	-581 (6)	158 (4)	792 (8)	5.0 (1)	6.4 (1.3)
H(C8)	-331 (6)	274 (5)	765 (7)	5.0 (1)	6.7 (1.3)
H(C9)	-245 (5)	370 (4)	448 (7)	4.0 (1)	5.5 (1.3)
H'(C9)	-123 (5)	338 (4)	611 (7)	5.0 (1)	6.3 (1.3)
H(C11)	55 (6)	252 (5)	-127 (8)	5.0 (0)	8.9 (0.0)
H(C12)	20 (7)	83 (5)	-104 (9)	6.0 (1)	8.2 (1.7)
H(C14)	-173 (4)	117 (3)	498 (5)	3.0 (1)	4.0 (0.9)
H(C16)	-415 (8)	7 (5)	4 (11)	9.0 (0)	11.1 (0.0)
H'(C16)	-593 (6)	4 (5)	92 (8)	7.0 (1)	8.8 (1.7)
H''(C16)	-492 (12)	-89 (7)	56 (13)	12.0 (0)	14.6 (0.0)
H(C17)	-504 (5)	326 (4)	237 (6)	5.0 (1)	6.5 (1.3)
H'(C17)	-521 (5)	202 (4)	176 (7)	6.0 (1)	7.0 (1.4)
H''(C17)	-703 (5)	266 (4)	255 (6)	5.0 (1)	6.4 (1.2)
H(O10)	-7 (7)	407 (4)	7 (8)	5.0 (1)	6.2 (1.3)

Arnebinol (NS-4:2) Colourless needles, mp 163–164 °C (MeOH-water). HR MS: Calcd for C₁₆H₂₀O₂ 244.1464, Found 244.1428. UV λ_{max}^{EtOH} nm (log ϵ): 213 (4.42), 294.2 (3.83). ¹H-NMR (CDCl₃, 400 MHz) and ¹³C-NMR (CDCl₃, 100 MHz) data are in Table I. MS m/z (rel. int.): 244 (M⁺, 65.3), 229 (14.4), 161 (84.6), 121 (100), 55 (51.2). Inhibition of PG biosynthesis: 75.0% (20 μ g/ml); IC₅₀=29.5 μ M.

Crystal Data of Arnebinol (2) Space group: $P2_1$. a =7.633 (4), b =13.868 (7), c =6.300 (3) $\text{\AA}$, β =92.98 (5), Z =2, D_x =1.11 g cm⁻³, V =666.0 $\text{\AA}^3$.

X-Ray Analysis of Arnebinol (2) All reflection data was recorded on a Philips PW 1100 four circle diffractometer with graphite monochromated CuK α radiation. A total of 1315 reflection was recorded within a range of 3–78°. The structure was solved by the direct method (MULTAN) and refined by block-diagonal least-squares (BLS). A final R value was 0.052 with anisotropic temperature factors for 16 carbon and 2 oxygen atoms, and isotropic temperature factors for 20 hydrogen atoms.

Des-O-methylasiodiplodin (NS-7:4) Colourless needles of mp 128–129 °C (MeOH-water). HR MS: Calcd for C₁₆H₂₂O₄ 278.1519, Found 278.1507. UV λ_{max}^{EtOH} nm: 224, 303. IR ν_{max}^{KBr} cm⁻¹: 3340, 1617, 1580, 1460, 1380, 1260, 1140, 1102, 1087, 1013, 920, 837, 713. ¹H-NMR (CDCl₃,

100 MHz) δ : 1.31 (3H, d, $J=6$ Hz, C-9'), 1.34—1.67 (10H, m, C-2'-C6'), 1.71—1.86 (2H, m, C-7'), 2.43, 3.21 (1H $\times$ 2, m $\times$ 2, C-1'), 5.11 (1H, ddq, $J=8, 6, 1.5$ Hz, C-8'), 5.34 (1H, brs, OH), 6.16 (1H, d, $J=2.5$ Hz, C-3), 6.21 (1H, d, $J=2.5$ Hz, C-5), 12.0 (1H, brs, OH). ^{13}C -NMR (CDCl_3 , 25 MHz) δ : 20.0 (C-9'), 21.1, 24.0, 24.5, 27.1, 30.7 (C-2'-C-6'), 31.0 (C-7'), 33.4 (C-1'), 75.0 (C-8'), 101.3 (C-3), 105.5 (C-1), 110.6 (C-5), 149.3 (C-6), 159.9 (C-2), 165.3 (C-4), 171.7 (C-7).

Acknowledgement We acknowledge the Ministry of Education, Science and Culture Japan for providing Grants-in-Aid. We also wish to thank Dr. D. C. Aldridge for an authentic sample of des-*O*-methyllasiiodiplodin and Prof. K. Mori of the University of Tokyo for the supply of synthetic samples of arnebinol and isoarnebinol.

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