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STRUCTURE OF ARNEBINOL, A NEW ANSA-TYPE MONOTERPENYLBENZENOID WITH INHIBITORY EFFECT TO PROSTAGLANDIN BIOSYNTHESIS

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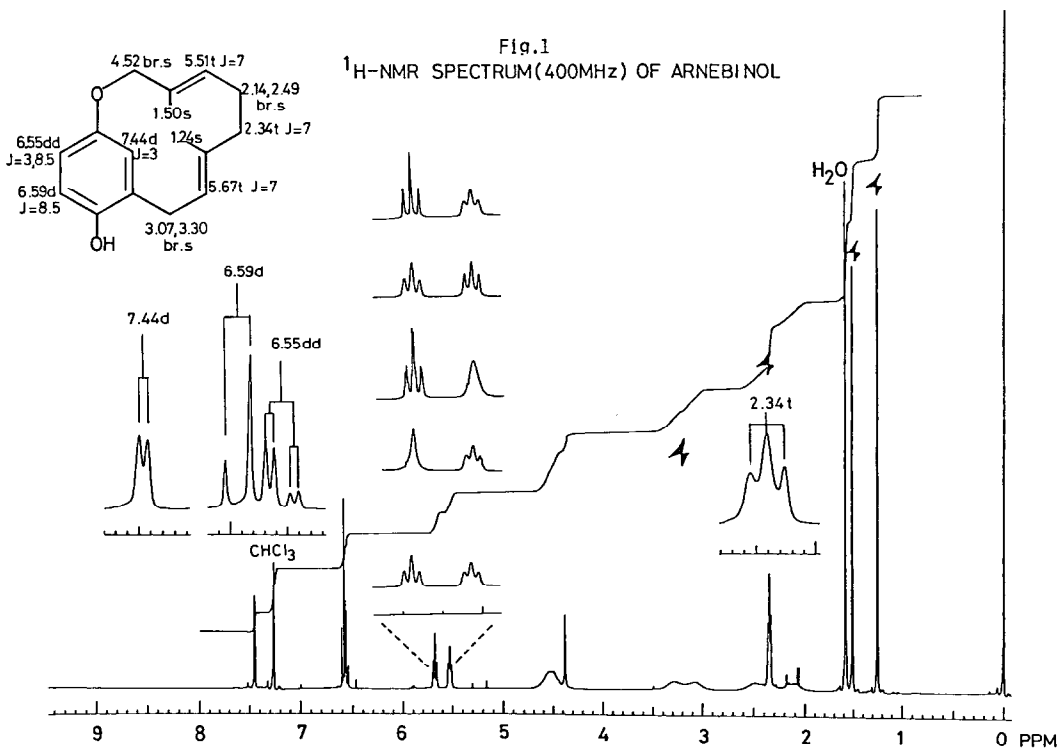
Summary: The two inhibitors of prostaglandin biosynthesis were isolated from the root of Arnebia euchroma (Boraginaceae). A colourless crystalline compound was identified to be a polyketide, des-O-methyllasiodiplodin (**3**) formerly obtained from a fungus, Lasiodiplodia theobromae. The structure of the other colourless crystalline compound, named arnebinol (**1**), was deduced from spectral investigations to be a dehydroderivative of geranylhydroquinone and finally determined by X-ray analysis to be a novel ansa-type monoterpenylbenzenoid.

Since Vane found aspirin like drugs exhibiting their antiinflammatory effect by the inhibition of fatty acid cyclooxygenase, an enzyme catalyzing the first step of prostaglandin(PG) biosynthesis,[2] extensive studies have been made on arachidonate metabolism including PG biosynthesis. It is now well established that arachidonate cascade provides classical PGs, PGI_2 (prostacyclin)[3], thromboxane (TX)[4], leukotriene (LT)[5] and metabolites of lipoxygenases (HPETE and HETE)[6]. They play a large variety of fundamental physiological actions in various organs and tissues. The compounds which inhibit or activate enzymes involved in arachidonate cascade are expected to have a variety of pharmacological effects. We have introduced a PGE_2 synthesizing enzyme system as a bio-assay test in our studies to find biologically active natural products from traditional medicinal drugs. In previous papers, we reported the inhibition of PG biosynthesis by different classes of compounds[7] and based on the study of structure activity relationships of metha-depsides and related compounds, we proposed a new active site model of fatty acid cyclooxygenase[8]. This paper reports the identification and structural elucidation of two inhibitors of PG biosynthesis contained in the root of Arnebia euchroma (Royle) Johnst., Japanese name "Nan Shikon", which has been used as a medicinal drug in Chinese medicine.[9] Hot aqueous extracts of this plant showed 39.3% inhibition to a PG

biosynthesizing enzyme system[8] at a concentration of 750 µg/ml. The main constituents of A. euchroma are naphthoquinone congeners,[10] however the hot aqueous extracts showing inhibitory effect to PG biosynthesis did not contained naphthoquinone pigments and the inhibitors of PG biosynthesis were thought to be other compounds. The inhibitors were found to be extracted by methanol and after several chromatographies of methanolic extracts on silica gel and Lober(RP-8) columns a colourless crystalline compound, named arnebinol (**1**), was isolated as an inhibitor of PG biosynthesis (IC_{50} 29.5 µM).

Arnebinol (**1**) gave following physical and spectral data; m.p. 163.5-164° C, High resolution MS Calcd for $C_{16}H_{20}O_2$ m/z 244.1464: Found m/z 244.1428; UV λ_{max}^{EtOH} nm (log ε): 213.2(4.42) and 294.2(3.83). The ^{13}C-NMR spectrum (100.7 MHz) revealed the presence of 10 aromatic and olefinic carbons, and the ^{13}C-NMR spectrum measured under INEPT pulse sequence and ^{1}H-NMR (400 MHz) spectra showed the presence of four aliphatic methylene, two olefinic methyl and two olefinic methine groups. The ^{1}H-NMR spectrum clearly indicates the presence of three aromatic protons of a 1,2 and 4 substituted benzene as it appears in Fig. 1. Successive decoupling experiments revealed that two olefinic methine protons coupled to aliphatic methylene groups, and at the same time showed long range couplings with respective methyl groups. Of the four aliphatic methylene groups, only one showed a clear triplet (δ 2.34), 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). These spectral investigations and the fact that geranylhydroquinone is a common precursor of the constituents of Boraginaceae plants,[11] led to the conclusion that arnebinol (**1**) was a

Fig.1
^{1}H-NMR SPECTRUM(400MHz) OF ARNEBINOL



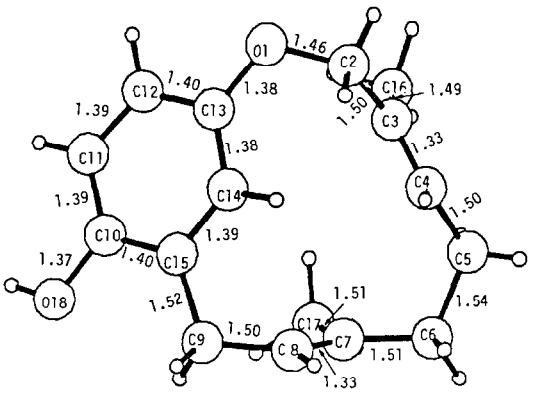
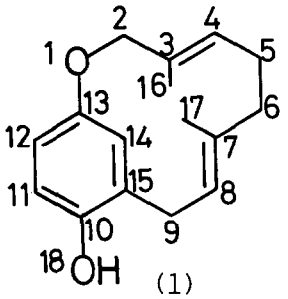


Fig. 2 PLUTO drawing of arnebinol (1)

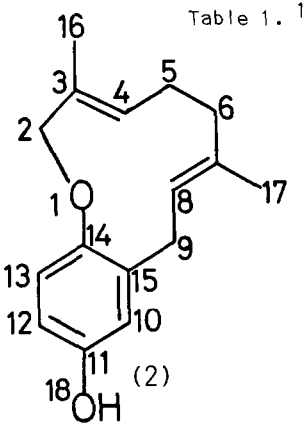
No. of Atoms	^{1}H-NMR (δ)	^{13}C-NMR (ppm)
1	-	-
2	4.52 (2H, br.s)	-[a]
3	-	140.2
4	5.51 (1H, br.t, J=7 Hz)	127.0
5	2.14, 2.49 (2H, br.s X 2)	25.3 or 25.8
6	2.34 (2H, t, J=7 Hz)	25.3 or 25.8
7	-	133.6
8	5.67 (1H, br.t, J=7 Hz)	124.2
9	3.07, 3.30 (2H, br.s X 2)	39.3
10	6.59 (1H. d. J=8.5 Hz)	118.2
11	6.55 (1H, dd, J=3, 8.5 Hz)	115.1
12	-	151.8
13	7.44 (1H, d, J=3 Hz)	115.2
14	-	151.8
15	-	146.8
16	1.50 (3H, s)	14.8
17	1.24 (3H, s)	12.6
18	4.38 (1H, s)	-

a. Overlapped with solvent signal.

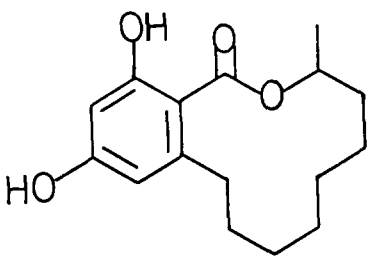
Table 1. ^{1}H and ^{13}C-NMR of arnebinol(**1**).



(1)



(2)



(3)

dehydroderivative of geranylbenzhydroquinone, in which pro-Z terminal methyl group formed an ether linkage with one of the hydroxy groups of hydroquinone. This leaves two possible structures, **1** and **2**, for arnebinol. The monoterpenyl moiety of **2** forms a 11 membered ring, while that of **1** is a 12 membered ring. All the methylene groups of 11 membered ring sesqiterpene, humulene, showed rather sharp signals in ^{1}H-NMR spectrum (400 MHz), indicating that arnebinol is preferably represented by structure **1**, in which 12 membered ansa-ring causes a strong hinderance in free rotation of three methylene groups, resulting that equilibrium between conformational isomers fall in a ragne of NMR time scale to give two pairs of extremely broad singnals for C-5 and C-9, and a broad singlet for C-2. Finally the structure of arnebinol (**1**) was unambiguously established by X-ray analysis.

Crystal of arnebinol (**1**) obtained from benzene is monoclinic, space group $P2_1$ with two molecules in a cell of dimensions, a=7.633(4), b=13.868(7), c=6.300(3) Å, β=992.98(5)°, Dx=1.11 g cm^{-3} and V=666.0 Å^3. All the reflection data were recorded on a Philips PW 1100 four circle diffractometer with graphite monochromated CuKα radiation. A total of 1315 reflections were

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 oxygen atoms, and isotropic temperature factors for 20 hydrogen atoms.

The othr colourless compound of m.p. 128.5-129.5° possessing a strong inhibitory effect to PG biosynthesis was also isolated after repeated chromatographies on silica gel, Lobar (RP 8) and LH-20 columns. The molecular formula was determined to be $C_{16}H_{22}O_4$ by high resolution MS. The ^{1}H-NMR spectrum revealed the presence of two meta-coupled aromatic protons, two phenolic hydroxy groups, a methyl group giving a doublet signal and a methine group bonded to the methyl group and an oxygen atom. The INEPT ^{13}C-NMR spectrum revealed that the compound contained 7 methylene groups. It was finally identified as des-O-methyllasiodiplodin (**3**), which had been isolated from a fungus Lasiodiplodia theobromae, by direct comparison with an authentic sample. Inhibition % of PG biosyntheis by des-O-methyllasiodiplodin (**3**) was 62.7% at a concentration of 20 g/ml. It is not clear whether des-O-methyllasiodiplodin (**3**) is produced by the plant, by a fungus contaminating the plant or by a fungus symbiotically grown in the plant. It is of interest that a space filling model (CPK model) of des-O-methyllasiodiplodin (**3**) is very similar to that of arnebinol (**1**).

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