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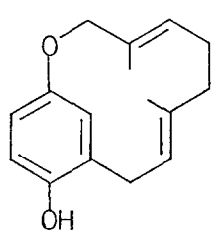
STRUCTURE OF ARNEBINONE, A NOVEL MONOTERPENYLBENZOQUINONE WITH INHIBITORY EFFECT TO PROSTAGLANDIN BIOSYNTHESIS

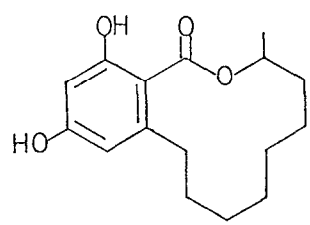
Yao Xin-Sheng[1], Yutaka Ebizuka, Hiroshi Noguchi, Fumiyuki Kiuchi and Ushio Sankawa*
Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan
Haruo Seto
Institute of Applied Microbiology, University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo 113, Japan

Summary: Two compounds possessing inhibitory effect against a prostaglandin synthesizing enzyme system were isolated from the root of Arnebia euchroma. A slightly yellow coloured oil was identified as a mixture of shikonofuran B and C (**3**), which had been isolated from Lithospermum erythrorhizon. The structure of the other orange compound, named arnebinone (**4**), was elucidated by spectroscopic studies to be a novel monoterpenylbenzoquinone, in which the monoterpenyl moiety formed a condensed six membered ring to a benzoquinone. The monoterpenyl moiety has a methyl, a vinyl and an isopropiridene groups. The structure of arnebinone (**4**) indicates that it is presumably biosynthesized via successive migration reactions from geranylhydroquinone (**5**), the common intermediate of naphthoquinone congeners, arnebinol (**1**) and shikonofuran (**3**).

As a continuation of our studies on the modulators of arachidonate cascade,[2] in a previous paper we reported the isolation and structural elucidation of two potent inhibitors of prostaglandin (PG) biosynthesis from the root of Arnebia euchroma (Royle) Johnst., Japanese name "Nan Shikon", which has been used as a medicinal drug in Chinese medicine.[3] One of the



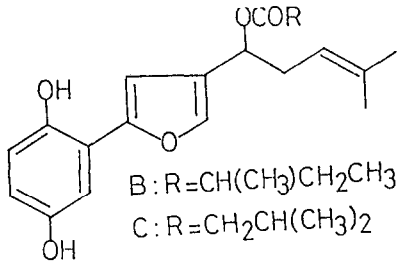
(1)



(2)

inhibitors was a novel ansa-type monoterpenylhydroquinone derivative, named arnebinol (**1**), and the other was a known polyketide, des-O-methyllasiodiplodin (**2**), first isolated from a fungus, Lasiodiplodia theobromae.[4] This paper reports the isolation of another two inhibitors of PG biosynthesis from the same plant.

After repeated chromatographies on silica gel and Lobar (RP-8) columns, a slightly yellow coloured oil exhibiting a strong inhibitory effect to a PG synthesizing enzyme system was isolated from the methanolic extracts of this plant. It was rather unstable and tended to give a coloured non-polar compound, indicating that it had a hydroquinone structure. High resolution MS (Calcd. for $C_{21}H_{26}O_5$ m/z 358.1779; Found m/z 358.1760) and the ^{1}H-NMR (100 MHz) spectrum suggested that it was a mixture of shikonofuran B and C (**3**), which had been isolated from the root of Lithospermum erythrorhizon.[5] In order to determine the composition of fatty acids, the mixture of shikonofurans was treated with 3% anhydrous HCl-MeOH at 100° for 3 hr in a sealed tube. The mixture of fatty acid methyl esters was extracted with n-hexane after dilution with water. GC-MS (Shimadzu-LKB 9000B with PAC 300 computer system and DEGS GLC column) revealed that the fatty acid methyl esters consisted of methyl α-methylbutyrate and isovalerate in a ration of 1.4:1.0. This indicate the obtained shikonofurans being the mixture of shikonofuran B and C (**3**) in a ratio of 1.4:1.0. The shikonofuran mixture showed 72.5% inhibition of PG biosynthesis at a final concentration of 20 μg/ml in our assay system.[6]



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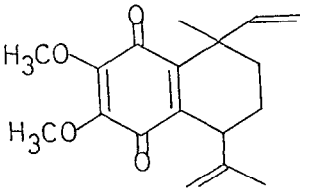
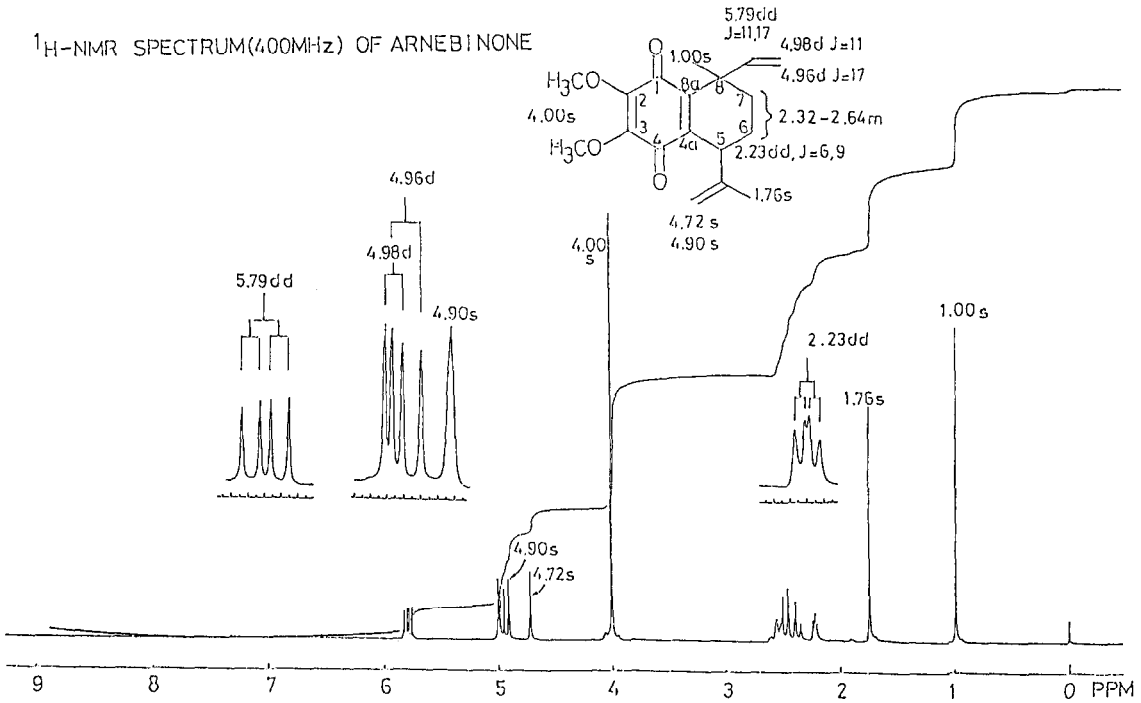
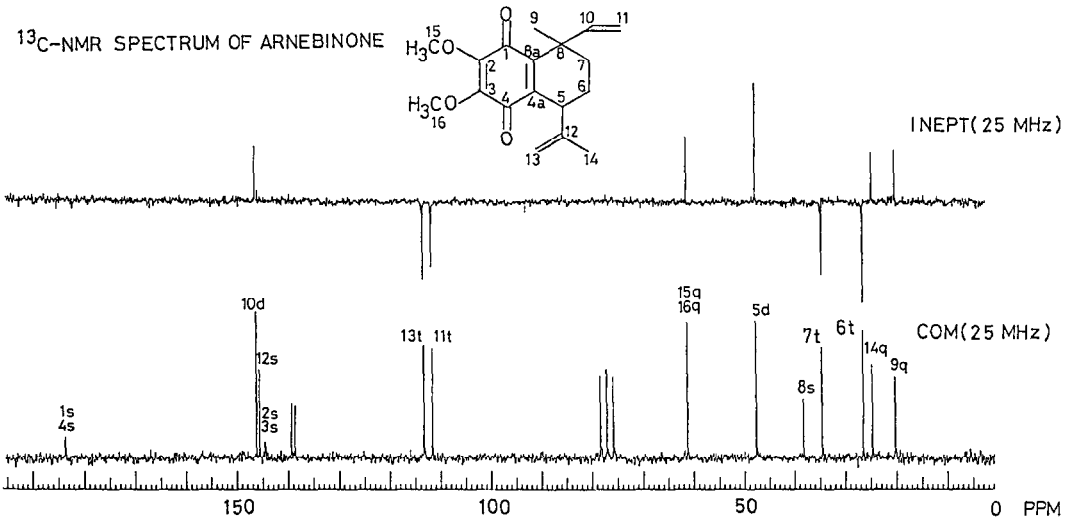


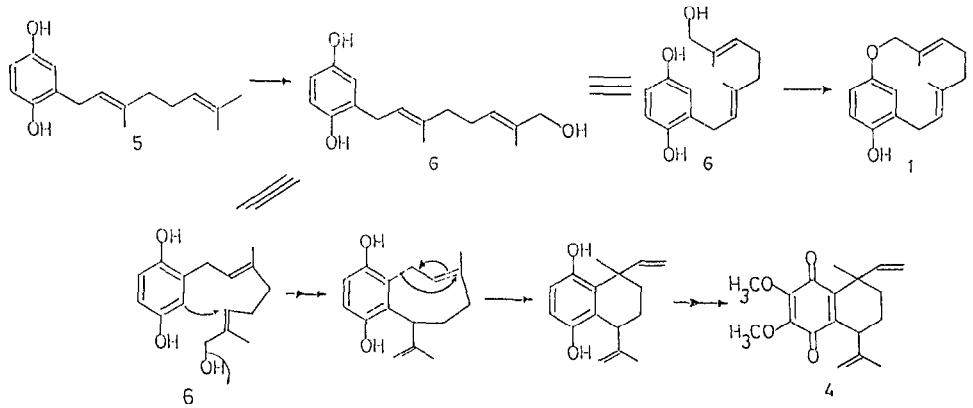
(4)

*The other inhibitor, named arnebinone (**4**), was isolated as an orange oil.* It showed 24.5% inhibition of PG biosynthesis at a concentration of 20 μg/ml. When arnebinone (**4**) was kept at a low temperature, it solidified to give yellowish orange crystals, however many attempts of recrystallization were unsuccessful. Arnebinone (**4**) gave following physical and spectral data; High resolution MS: Calcd for $C_{18}H_{22}O_4$ m/z 302.1518: Found 302.1518; UV λ_{max}^{EtOH} nm (log ε): 207 (4.13), 277 (4.11) and 410 (2.76); $[\alpha]_D^{20°}$ -4° (c=0.01, dioxane). The presence of benzoquinone skeleton was easily deduced from the UV absorption spectrum.[7] The ^{1}H-NMR spectrum (400 MHz) indicated the presence of an aliphatic and an olefinic methyl groups and two methoxyl grups giving a 6H singlet siganl. In the olefinic region arnebinone (**4**) showed typical ABX signals; δ 4.96 (d, J=17 Hz), 4.98 (d, J=11 Hz) and 5.79 (dd, J=11, 17Hz); indicating the presence of a vinyl group bonded to a quarternary carbon. Two singlet signals at δ 4.72 and 4.90 indicated arnebinone (**4**) contained a vinilidene group. The presence of the vinyl and vinilidene grups was further supported by the ^{13}C-NMR spectrum (25 MHz) measured with a INEPT pulse sequence. A





methine signal observed at δ 2.23 as a double-doublet of J=6 and 9 in the ^{1}H-NMR spectrum indicated that the methine group should be accommodated adjacent to an aliphatic methylene group. From the NMR spectra it is quite evident that all the six carbons of the benzoquinone are substituted. The ^{13}C-NMR spectrum showed the presence of another quarternary carbon (38.1 ppm). The spectral data and biosynthetic consideration led us to the conclusion that arnebinone (**4**) should be represented by the sturcture **4**, which satisfied all the requirements of spectral data.



Hyptothetical biosynthetic scheme of arnebinol(1) and arnebinone(2)

Arnebinone (**4**) is a novel monoterpenylbenzenoid derived from geranylhydroquinone (**5**), the common intermediate of various metabolites of Boraginaceae plants.[8] The scheme illustrates possible biosynthetic reactions starting from geranylhydroquinone (**5**) to give arnebinol (**1**) and arnebinone (**4**), in which alliodorol (**6**) is the common intermediate of arnebinol (**1**) and arnebinone (**4**).

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