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Note

High-performance liquid chromatographic determination of the sweet component in sweet hydrangea

YOHEI HASHIMOTO, MASATAKA MORIYASU*, YOSHIE KANAI and MASAKO HIRASE
Kobe Women's College of Pharmacy, Motoyamakita-machi, Higashinada-ku, Kobe, 658 (Japan)
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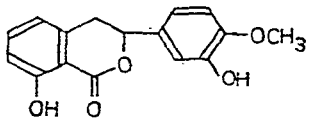
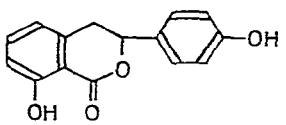
Sweet hydrangea (Japanese name: Amachya) is a crude drug which generally consists of dried leaves of a cultivated species, *Hydrangea serrata* Seringe var. *Thungerii Sugimoto* (Saxifragaceae). It is sometimes prepared from some wild *Hydrangea* species. The crude drug has a strong, sweet taste due to an isocoumarin derivative, phyllodulcin (I)[1,2], derived by hydrolysis of its glycoside. The intensity of this sweet taste is at least 50 times greater than that of sucrose. A similar isocoumarin derivative, hydrangenol (II), which does not have a sweet taste, is also found in this crude drug[3].



I



II

There have been only a few reports on the analysis of this crude drug, including quantitative thin-layer chromatography, reported elsewhere by Hashimoto and co-workers[4,5]. In this paper, the quantitative analysis of I by high-performance liquid chromatography (HPLC) is described.

EXPERIMENTAL

The HPLC apparatus was of our own construction. The analysis of the chromatographic data was performed with a Chromatopak E 1A (Shimadzu, Kyoto, Japan), retention times and peak areas being recorded. The column was a Shodex B-804 gel-permeation type (Showa Denko, Minatoku, Tokyo, Japan), 50 cm × 8 mm I.D., which is suitable for the separation of water soluble organic substances. Detection was effected with a UV detector at 314 nm, which corresponds to the absorption maximum of I ($\log \varepsilon = 3.70$).

Standard samples of I (which was obtained from sweet hydrangea by the authors), II, rutin, quercetin, umbelliferone, kaempferol and *p*-hydroxybenzaldehyde were used. The presence of the last six substances in the crude drug has been reported[6].

The sample for HPLC was prepared from the crude drug as follows. The plucked leaves were dried in the sun for 2 days, sprinkled with water and then dried again

* To whom correspondence should be addressed.

for 1 day. The dried leaves were crushed in a mortar and 0.5 g of the powder was weighed out. Extraction was carried out four times with 40 ml of methanol for 30 min and the solvent was removed from the combined extracts by distillation A 30% aqueous solution of tetrahydrofuran (THF) was added to the residue and the volume of the solution was made up to exactly 30 ml with 30% THF. After standing for 2 h, the solution was filtered and then used for HPLC analysis.

RESULTS AND DISCUSSION

Standard samples of each substance and the extract from sweet hydrangea were chromatographed using different aqueous eluent systems. The presence of an organic solvent miscible with water, such as methanol, THF, acetonitrile or dioxane, was necessary for elution. The retention times of each substance increased as the water content in the eluent decreased. Of the solvent systems examined, water–THF gave the shortest retention times.

The retention times also depended on the pH of the eluent. With a decrease in pH, the retention times increased, and in neutral solution the retention times of I and II were fairly long. Further, in neutral borate buffer, the retention times of I and II were close to each other, that of the latter being slightly greater. Therefore, a weakly a weakly basic solution was most favourable.

Fig. 1 shows an exanple of the chromatograms of the extract. The retention times of the standard samples under the conditions used were 13.5 min (phyllodulcin, I), 7.3 min (hydrangenol, II), 7.3 min (umbelliferone), 6.8 min (quercetin), 7.2 min (*p*-hydroxybenzaldehyde), 6.8 min (kaempherol) and 6.7 min (rutin). All of the compounds except I, therefore, should be eluted in the region of peaks 1 and 2 in Fig. 1.

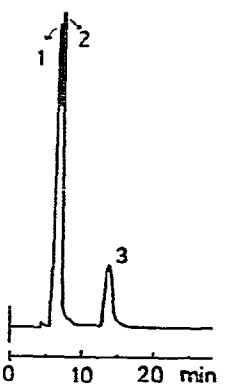


Fig. 1. Chromatogram of extract of sweet hydrangea. Peaks: 1 and 2, see text; 3, phyllodulcin (I). Conditions: column, Shodex B-804; eluent, THF–water (30:70) + 0.33% sodium borate; flow-rate, 1.5 ml/min.

In comparison with borate buffer, the use of phosphate buffer as the eluent sometines gave different retention times under otherwise identical conditions (pH, THF content). For example, whereas the retention time of I was only slightly affected by the type of buffer, the retention time of II in phosphate buffer was so long that the peaks of I and II almost overlapped. This phenomenon might be attributable to the different complex formation properties of these substances with borate.

The determination of I in different sweet hydrangea samples was carried out under the condition shown in Fig. 1 and the results are given in Table I. The calibration graph of peak area *versus* amount of sample tested (up to 10 μg) was linear. The detection limit was about 0.2 μg. The coefficient of variation was 3.0% for sample c in Table I (six measurements).

TABLE I

CONTENTS OF PHYLLODULCIN (I) IN THE LEAVES OF SWEET HYDRANGEA

Sample	*Botanical name*	*Phyllodulcin content (%)*
a	*Hydrangea serrata* Seringe var. *Thungerii Sugimoto*	3.0
b	*Hydrangea serrata* Seringe var. *Thungerii Sugimoto*	1.4
c	*Hydrangea serrata* Seringe	4.4
d	*Hydrangea serrata* Seringe	3.1
e	*Hydrangea serrata* Seringe	0.9
f	*Hydrangea macrophylla* (Thunb.) Seringe var. *macrophylla*	0.9

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