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CHEMOTAXONOMIC SIGNIFICANCE OF *ENT*-KAURENE DITERPENES IN *RABDOSIA UMBROSUS* VARIETIES

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Key Word Index—*Rabdosia*; Labiatae; *ent*-kaurene diterpenes; single leaf analysis; reverse phase HPLC.

Abstract—Quantitative identification of six *ent*-kaurene diterpenes, by reverse phase HPLC, in crude ether extracts of a single leaf of five major *Rabdosia umbrosus* varieties is described. These diterpenes are significant chemosystematic markers among these plants.

INTRODUCTION

Due to the confusion in the taxonomy of *Isodon* plants, Hara recently established a new system of classification for the Asiatic species which occur mainly in Himalaya, Thailand, China and Japan [1]. He reclassified numerous genera, species and subspecies, formerly designated as *Plectranthus*, *Isodon*, *Amethystanthus* and *Homalocheilos*, as 118 new combinations of *Rabdosia* (Labiatae).

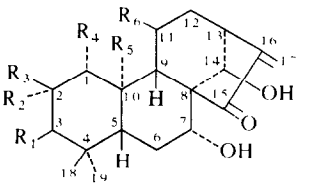
In recent years *Rabdosia* plants have been extensively screened for biologically active diterpenes [2]. These diterpenes are common chemical constituents of this genus, and therefore, as a result, they may clarify the confused taxonomy of *Rabdosia* plants. The present investigation considerably extends the knowledge of these diterpenes in order to evaluate their usefulness for taxonomic purposes.

RESULTS AND DISCUSSION

Recently we reported that HPLC is an efficient and convenient method to identify *Rabdosia* diterpenes upon direct injection of the crude MeOH extract prepared from only one dried leaf into the reverse phase C_{18} column [3]. Application of this HPLC method to five varieties of *R. umbrosus*; namely *R. umbrosa* Maxim. var. *umbrosa* Hara, *R. umbrosus* var. *latifolia* (Okuyama) Hara, *R. umbrosus* var. *excisinflexa* (Nakai) Hara, *R. umbrosus* var. *hakusanensis* (Kudo) Hara and *R. umbrosus* f. *Kameba* (Okuyama ex Ohwi) Hara has resulted in the identification of a total of six *ent*-kaurene diterpenes. Of these five plants, the chemical constituents of both *R. umbrosus* var. *hakusanensis* (Kudo) Hara and *R. umbrosus* var. *latifolia* (Okuyama) Hara have not been reported previously.

These five are major varieties of this species found in Japan. Morphological identification of these varieties is difficult, especially before their flowering when it is almost impossible. The *ent*-kaurene diterpenes identified within the range of the applied HPLC conditions in these plants are kamebakaurinin **1** [4], isodomedin **2** [5], umbrosin A **3** [6], mebadonin **4** [7], kamebakaurin **5** [8], and kamebanin **6** [9] all of which were previously isolated from other *Rabdosia* plants as antitumor [10], antibacterial [11], and insect growth inhibitory [12] principles.

Kamebanin was noted in all varieties, accompanying at least one of the other members of *ent*-kaurene diterpenes. It is noteworthy that umbrosin A always co-occurred with the corresponding epimer, mebadonin. Kamebakaurin or isodomedin were found as the major constituent of two varieties, but do not seem to be common components in these plants. The most polar compound in this series is kamebakaurinin, which was observed in only two of the varieties.



	(a)	$R_1 = R_2 = R_3 = R_4 = R_6 = H$, $R_5 = Me$
1	Kamebakaurinin	$R_1 = R_2 = R_3 = R_4 = H$, $R_5 = CH_2OH$, $R_6 = OH$
2	Isodomedin	$R_2 = R_3 = R_6 = H$, $R_1 = OAc$, $R_4 = OH$, $R_5 = Me$
3	Umbrosin A	$R_1 = R_2 = R_4 = R_6 = H$, $R_3 = OH$, $R_5 = Me$
4	Mebadonin	$R_1 = R_3 = R_4 = R_6 = H$, $R_2 = OH$, $R_5 = Me$
5	Kamebakaurin	$R_1 = R_2 = R_3 = R_6 = H$, $R_4 = OH$, $R_5 = CH_2OH$
6	Kamebanin	$R_1 = R_2 = R_3 = R_6 = H$, $R_4 = OH$, $R_5 = Me$

The results are presented in Fig. 1. The relative abundance of *ent*-kaurene diterpenes was determined with respect to the major constituent of each variety. The results show clearly that the diterpene contents, which are significantly variable among the varieties of *R. umbrosus*, represent a taxonomically useful key character.

Although we have identified three *ent*-kaurenoid constituents in the crude MeOH extract of *R. umbrosus* var. *excisinflexus* [3], our further studies by HPLC indicates that Et_2O is a superior solvent, and that a simple semi-purification of this Et_2O extract is also advisable before HPLC. The biological activity known for *Rabdosia* diterpenes is attributed to the α-methylenecyclopentanone moiety, which in turn is the chromophore responsible for detection of these substances by HPLC [13]. The order of elution under the applied HPLC conditions was as follows; kamebakaurinin 5.5 min, isodomedin 7.4 min, umbrosin A 9.3 min, mebadonin 10.9 min, kamebakaurin 12.6 min and kamebanin 15.8 min. These *ent*-kaurenoids possess a common carbon skeleton **a**, *ent*-7α,14β-dihydroxy-kaur-16-en-15-one, but differ in their sites and levels of oxidation at C-1, C-2, C-3, C-11 and C-20.

The retention of these diterpenes is governed by the position and the stereochemistry of the additional OH group(s) to **a** as indicated by the following:

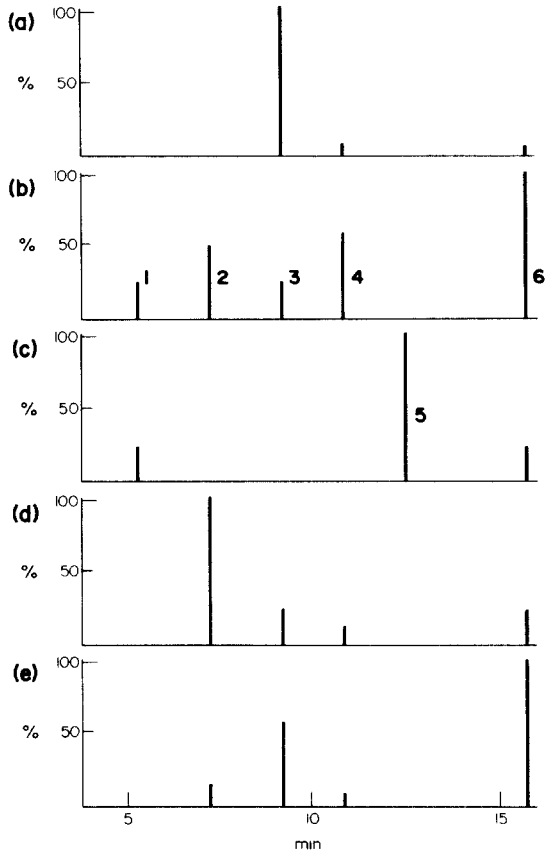


Fig. 1. Bar graph of HPLC showing relative abundance of *ent*-kaurene diterpenes in five varieties of *Rabdosia umbrosus*; (a) var. *umbrosa*, (b) var. *latifolia*, (c) var. *excisinflexa*, (d) var. *hakusanensis*, (e) f. *Kameba*.

1. Effect on polarity is greater when an additional OH group to **a** is situated at the C-2 rather than at the C-1 position; and of the two epimers at C-2, umbrosin A and mebadonin, the equatorial hydroxyl in **3** shows more contribution to polarity than the axial **4**.

2. An increase in the number of hydroxyl group(s), without consideration of their position in the molecule, does not necessarily mean an increase in polarity. Comparison of (**3**) with (**6**) shows that kamebakaurin **5**, which has two additional hydroxyl groups to **a**, one at C-1 equatorial and the other one at C-20 (angular axial-CH_2OH group) is more polar than kamebanin **6**, which has an additional C-1 equatorial hydroxyl to **a**, whereas **5** is less polar than mebadonin **4** or umbrosin A **3** which both possess one additional hydroxyl group to **a** at C-2.

3. Kamebakaurinin **1**, having one of the two additional hydroxyl groups at C-11 equatorial, is more polar than its counterpart kamebakaurin **5** possessing one at C-1 equatorial position.

Analysis of the diterpenes by HPLC provides a rapid and reliable means for the taxonomic classification of *Rabdosia* spp. according to their secondary metabolites. The use of minimal amounts of plant material, one leaf, will exclude any phytochemical confusion which could arise from a large collection of the samples. The reproducibility of the results with respect to site variation was checked. For instance, the same diterpene pattern resulted by HPLC with a single leaf of *R. umbrosus* Maxim. var. *umbrosa* Hara which was collected in August, 1980 in Miyagi-Sendai which is located about 200 miles north from Shizuoka-Amagi, the site of first collection. The concentration of diterpene constituents in each plant may vary seasonally, but such variations could easily be detected by HPLC.

EXPERIMENTAL

Plant materials. The five varieties of *Rabdosia umbrosus*, with the locality and date of collection, are: var. *umbrosa*, Shizuoka-Amagi, Aug. 1980; var. *latifolia*, Yamanashi-Misaka, June 1980; var. *excisinflexa*, Miyagi-Jogi, Sept. 1980; var. Toyama-Bijodaira, Sept. 1980; f. *Kameba*, Gunma-Shimonida, Aug. 1980.

Sample preparations. A single dried leaf (20–50 mg) of the plant was pulverized and extracted with Et_2O (5 ml). After filtration and removal of the solvent, the residue was dissolved in MeOH (3 ml) and passed through a short column packed with Si gel (0.5 cm), charcoal (0.2 cm), and Si gel (0.5 cm) respectively, in a 0.5-cm o.d. column, then washed with MeOH (2 ml). This MeOH solution was injected into HPLC without further purification.

HPLC. HPLC was carried out with a DuPont Model 850, liquid chromatograph on a prepacked reverse phase DuPont Zorbax ODS, 5–6-μm particle size, column of 4.6 mm (i.d.) × 25 cm dimensions. This column was protected with a pre-column of 2.1 mm (i.d.) × 7 cm, packed with pellicular Whatman Co:Pell ODS. The solvent system consisted of MeOH–H_2O (9:11) at a flow rate of 2 ml/min and at an average pressure of 179–180 bar. Effluents were detected with a DuPont spectrophotometer and micro-flow cell at 230 nm. Analytically pure diterpenes, available from previous studies, were used as internal standards. The solvent proportions and flow rate were adjusted so that the diterpenoids could be separated within a 16-min time span, with minimum background interference. For each plant profile, the peak area of the major component was considered as 100% and the peak area of the other components was measured and shown as a percentage of the major peak.

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REFERENCES

1. Hara, H. (1972) *Jpn. J. Botany* **42**, 193.
2. Kubo, I., Taniguchi, M. and Kubota, T. (1978) *Rev. Latinoam. Quim.* **9**, 158, and references therein.
3. Ganjian, I., Kubo, I. and Kubota, T. (1980) *J. Chromatogr.* **200**, 250.
4. Fujita, T., Ichihara, T., Takeda, Y., Takaishi, Y. and Shingu, T. (1978) Abstract paper of the 98th Regional Meeting of Japan Pharmaceutical Society, Okayama, p. 331.
5. Kubo, I., Miura, I., Nakanishi, K., Kamikawa, T., Isobe, T. and Kubota, T. (1977) *J. Chem. Soc. Chem. Commun.* 555.
6. Kubo, I., Kamikawa, T., Isobe, T. and Kubota, T. (1974) *Bull. Chem. Soc. Jpn* **47**, 1277.
7. Hirotsu, K., Isobe, T., Kamikawa, T., Kubota, T. and Shimada, A. (1973) *Chem. Letters* 255.
8. Fujita, T., Ichihara, T., Takeda, Y., Takaishi, Y., Minato, Y. and Shingu, T. (1977) Abstract papers of the 21st Symposium on Essential Oils and Terpenes, Tokushima, p. 24.
9. Kubo, I., Miura, I., Kamikawa, T., Isobe, T. and Kubota, T. (1977) *Chem. Letters* 1289.
10. Fujita, E., Nagao, Y. and Node, M. (1976) *Heterocycles*, **5**, 793, and references therein.
11. Kubo, I., Taniguchi, M., Satomura, Y. and Kubota, T. (1974) *Agric. Biol. Chem.* **38**, 1261.
12. Taniguchi, M., Yamaguchi, M., Kubo, I. and Kubota, T. (1979) *Agric. Biol. Chem.* **43**, 71.
13. Trivedi, G. K., Kubo, I. and Kubota, T. (1979) *J. Chromatogr.* **179**, 219.