Chemotaxonomical Studies of the Leaf Oils of *L. umbellata* Thunb.

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(Z. Naturforsch. 28 c, 227–228 [1973]; received December 6, 1972)

Chemotaxonomy, monoterpenes

L. umbellata Thunb. (Kuromoji in Japanese) which belongs to the *Lauraceae* family, is deciduous shrub and grows on the mountainous distinct all over Japan, The twigs have been used for the tooth picks since they have a fragrant odor. Before World War II, the essential oil (Kuromoji-yu) had been used for the perfume of the soap because it contains large amounts of linalool. The oil, however, has not been isolated at present.

Kuromoji is sometime divided into several varieties on the basis of morphological differences. According to the expert opinion of an authoritative, Kuromoji divided into several species and their subspecies.

Kuromoji
1. Kuromoji- *L. umbellata* Thunb.,
   Himekuromoji- *var. membraceae* (Maxim.) Momiyama,
   Obakuromoji- *var. lancea* Momiyama.
2. Kekuromoji- *L. sericea* (Sieb. et Zucc.) Blume,
   Usugekuromoji- *var. glabrata* Blume.

The morphological distinction of these five species, however is not clear. In the previous communication[1], we reported the chemotaxonomy of Kuromoji, Kekuromoji, and Usugekuromoji. In addition to the paper, the leaf oils of Himekuromoji and Obakuromoji belonging to the subspecies of Kuromoji were examined from the view point of chemotaxonomy.

The leaf oils were isolated from the fresh leaves by steam distillation. The main individual terpene constituents were isolated by column chromatography followed by preparative gas chromatography and identified by IR spectrum and by gas chromatography by comparison with authentic specimens.

The results of the investigation are shown in Table I. The percentage of the constituents was calculated from the areas of the peaks of gas chromatograms. The main constituents of the plants are following: Kuromoji 1,8-cineole and limonene 37.3 %, linalool 28.6%; Himekuromoji 1,8-cineole and limonene 29.2%, linalool 18.2%, carvone 23.7%; Obakuromoji 1,8-cineole and limonene 35.9 %, linalool 22.9%, caryophyllene 7.8%. We named those three Kuromojis Linalool-kuromoji, Carvone-kuromoji, and Caryophyllene-kuromoji respectively on the basis of the characteristic constituents. It seems reasonable that Carvone-kuromoji derived from Linaloolkuromoji by the biogenetic oxidation of limonene to carvone.

Table I. The compositions of the essential oils of *L. umbellata* Thunb. A: Kuromoji; B: Himekuromoji; C: Obakuromoji D: Kekuromoji; E: Usugekuromoji.

Compound	t_R [min]	Samples A	B	C [%]	D	E
α-pinene	2.5	7.1	5.1	6.0	11.2	11.1
camphene	2.7	4.4	3.3	3.0	5.1	10.8
β-pinene	3.1	1.3	0.8	1.0	3.6	5.8
Δ^3-carene	3.3	0.7	1.0	1.0	1.6	1.2
myrcene	3.5	1.6	1.4	1.1	2.0	—
limonene 1,8-cineole	3.9	37.3	29.2	35.9	50.3	3.3
γ-terpinene	4.4	4.9	2.2	4.0	2.6	1.4
linalool	5.3	28.6	18.2	22.9	7.1	4.2
unknown	5.9	0.7	0.3	—	—	—
borneol	6.5	0.5	0.4	0.8	0.6	—
unknown	6.8	2.6	1.9	3.6	1.8	—
unknown	7.1	3.2	3.3	3.7	6.1	0.5
carvone	8.1	—	23.7	0.5	0.6	0.2
unknown	8.3	1.6	—	—	—	—
geraniol	8.6	1.3	3.2	0.8	0.6	0.3
bornylacetate	9.3	0.6	0.4	0.8	1.0	6.7
unknown	10.1	1.6	—	—	—	1.8
unknown	10.3	—	0.1	—	—	—
unknown	10.7	—	—	1.5	—	1.2
geranylacetate	11.4	1.3	1.1	0.5	3.2	2.0
unknown	11.5	—	—	—	—	6.2
unknown	11.8	—	0.4	0.5	—	2.6
caryophyllene	12.4	0.2	1.1	7.8	1.2	13.3
unknown	13.2	—	0.3	0.8	—	—
unknown	13.8	—	0.9	—	—	—
unknown	14.0	0.2	1.1	2.5	1.0	1.9
unknown	15.1	—	—	—	—	0.6
cadinene	15.3	0.5	0.6	0.8	0.4	5.9
unknown	17.0	—	0.3	0.5	—	0.2
unknown	19.5	—	—	—	—	4.9
unknown	19.8	—	—	—	—	5.5
unknown	22.0	—	—	—	—	4.5
unkownn	23.0	—	—	—	—	2.0
unknown	23.5	—	—	—	—	2.5

As seen in Table I, the five species can be easily identified by means of chemical methods.

Isolation of the leaf oils. The fresh leaves cut in small pieces were subjected to steam distillation. The oils were extracted with ether and dried over anhydrous sodium sulfate. After the distillation of the ether, leaf oils were obtained (Fig. 1).

Sample A (Kuromoji): On September 7 in 1970, at Izunagaoka in Shizuoka Prefecture. 0.2 % yield, n_D^{25} 1.4658, α_D^{25} -17.0°.

Sample B (Himekuromoji): On October 14, 1971, at Shinkhiro in Aichi Prefecture. 0.4 % yield, n_D^{25} 1.4789, α_D^{25} +10.7°.

Sample C (Obakuromoji): On September 1, 1971, at Yonezawa in Yamagata Prefecture. 0.3% yield, n_D^{25} 1.4717, α_D^{25} -20.5°.

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The authors thank Dr. T. Seki (Department of Botany, Hiroshima University) for identification of the plants.

Fig. 1. Map of Investigation Areas (Japan).
● Caryophyllene-kuromoji;
○ Linalool-kuromoji;
▲ Carvone-kuromoji;
△ Kekuromoji;
◑ Usugekuromoji.

Sample D (Kekuromoji): On July 25, 1970, at Kuromoritoge in Ehime Prefecture. 0.23% yield, n_D^{25} 1.4646, α_D^{25} -16.0°.

Sample E (Usugekuromoji): On october 16 in 1971, at Yoshiwa in Hiroshima Prefecture. 0.08% yield, n_D^{25} 1.4873, α_D^{25} +27.4°.

The analysis of the oils by gas chromatography. For identification, gas chromatography was carried out with Hitachi Model K 53 gas chromatograph equipped with an ionization detector. The stainless steel column was packed with 5% SE-30 on Chromosorb W, temperature programmed from 70 to 250 °C.

[1] H. KOMAE, N. HAYASHI, S. KOSELA and T. ARATANI, Flavour Ind. **4**, 208 [1972].

Acid Hydrolysis of Colchicine and Related Compounds

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(Z. Naturforsch. 28 c, 228–230 [1973]; received November 20, 1972)

Colchicine, tropolones, kinetics

Acid-catalyzed hydrolysis of the methoxytropone moiety of colchicine has been shown to proceed at a greater rate than in the case of isocolchicine[1]. (Structures are shown in Table I.) No interpretation of this rate difference has been offered. And, while a mechanism for the acid-catalyzed hydrolysis of 2-methoxytropone has been proposed[2], no kinetic data on this type of reaction either for simple methoxytropones or on colchicine type compounds have been reported. Thus, the present investigation was undertaken to examine the effect of structural changes on the rate of acid hydrolysis of colchicine and to examine certain kinetic aspects of this reaction.

Experimental

Colchicine was purified according to ASHLEY and HARRIS[3]. Other compounds were prepared according to literature procedures (Table I).

The analytical procedure for unhydrolyzed methoxytropones was formulated to take advantage of the salt-forming ability of tropolones in alkaline media. A sample (1 ml) of the acidic reaction mixture was diluted with an alkaline buffer (0.1 M NaOH and 0.1 M NaH_2PO_4, pH = 11.8, 5 ml) and extracted with three, 3 ml portions of chloroform. The chloroform extract was diluted to a suitable concentration and the absorbance of the solution, corresponding to the concentration of the unhydrolyzed compound, was measured at the appropriate wavelength (Table I). All compounds were run at 75 °C in 0.127 M HCl containing 5% acetonitrile. Ionic strength was kept constant at 0.15 by addition of KCl. All reactions were followed to at least 75% completion.

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Results

Rate constants determined for the acid hydrolysis of the various compounds are shown in Table I and represent pseudo-first order rate constants obtained from the slopes of log-concentration-versus-time plots.

Hydrolysis rate constants for colchicine, isocolchicine and 2-methoxytropone were determined over a ten-fold range of hydrogen-ion concentration at constant ionic strength and are shown in Table II. The slope of a log k versus log $[H^+]$ plot was unity for each compound.

In order to determine the effect of an electron-withdrawing group on the rate of acid hydrolysis of colchicine, the 4-cyano derivative of colchicine was prepared. The cyano derivative was chosen since this substituent would be electron-withdrawing in the 4 position of colchicine which is *meta* to the bond joining the phenyl and tropolone rings. The rate of hydrolysis of 4-cyanocolchicine showed an increase over the rate of hydrolysis of colchicine.

Removal of the acetamido side chain from colchicine and isocolchicine, to give the corresponding desacetamido derivatives, was carried out to deter-