Notes

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Toshikazu Harada and Yasuhisa Saiki : Pharmaceutical Studies of Japanese Wild Ginger. II[1] Paper Chromatography of Essential Oils.

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A few studies have been presented on the chromatography of essential oils. Kirchner *et al.* reported the chromatostrip[2] in the case of microanalysis, and the chromatobar[3] for the method of purification, but paper chromatography was not hitherto successful. Furthermore, chromatostrip is available for microdetection but Rf values are apt to change, because it is difficult to obtain homogeneous strips. The writers tried paper chromatography by the use of treated paper and various solutions and successful separation was effected.

Paper—a) Toyo Roshi No. 2 (coarse filter paper) was dipped in 0.5% benzine solution of paraffine (m.p. 56~58°) and dried at room temperature.

b) The same paper was treated in 0.5% ether solution of cetaceum (purified spermaceti).

Solvents—Solvents must not dissolve paraffine and cetaceum, and therefore methanol, ethanol, acetone, acetic acid, ethyl acetate, etc. were used as solvents and their aqueous solutions. Methanol, hydrated acetone, hydrated ethanol, and hydrated acetic acid were found to be usable.

Color tests—Kirchner *et al.*[2] used bromine and fluorescein in the detection of a double bond and *o*-dianisidine for an aldehyde. The writers tested the following reagents : (a) Millon's reagent (1 part of Hg dissolved in 1 part of fuming HNO_3, and diluted with 4 parts of water) for phenols and phenol ethers; (b) Denigé's solution (5 g. of HgO dissolved in 20 cc. H_2SO_4 and diluted with 100 cc. water) for hydroxyl group; (c) 1% benzidine-HCl aq. solution; (d) 1% α-naphthylamine-HCl aq. solution; (e) 4% phloroglucinol-EtOH solution and conc. HCl solution; and (f) 1% aniline-HCl solution. Reagents (c), (d), (e), and (f) are known as reagents of lignin.[4]

Table I. Rf Value of Essential Oils

Compd.	Rf × 100				
	Par.	Cet.			
	MeOH	MeOH	50% Acetone	AcOH-H_2O 3:2	70% EtOH-NH_4OH*
Eugenol	65	85	98	75	85
Methyleugenol	72	68	87	70	95
Isoeugenol	75	83	95	68	95
Isomethyleugenol	70	70	60	80	97
Safrole	50	65	23	82	50
Asarone	75	82	90	90	99
α-*l*-Terpineol	85	88	85	70	95
Citronellol	75	83	68	83	95
Geraniol	70	92	88	92	90
Nerol	80	91	87	91	95
Linalool	70	85	70	85	95

One-dimensional ascending method.
Abbreviations : Par. : Paraffine treated. Cet. : Cetaceum treated.
* 95 cc. of 70% EtOH mixed with 5 cc. of 28% NH_4OH.

* Oshika, Shizuoka (原田利一, 斎木保久).

1) Part I : J. Pharm. Soc. Japan, **70**, 45 (1950). The previous title "Pharmacognostic Studies of Japanese Wild Ginger" is changed to the present one.
2) Kirchner, *et al.* : Anal. Chem., **23**, 420(1951).
3) J. M. Miller, Kirchner : *Ibid.*, **23**, 428(1951).
4) Hachihama, Johdai : "The Chemistry of Lignin," 35(1947).

Table II. Color of Spots

Compd.	Reagents (a)	(b)*	(c)	(d)	(e)	(f)
Eugenol	Br	V	Y	Y	R	Y
Methyleugenol	Or	V	Br	YW	RV	Y
Isoeugenol	Br	V	Y	Y	R	Y
Isomethyleugneol	Y	V	Y	YW	—	Y
Safrole	POr	V	Br	Br	RV	Y
Asarone	G	V	YBr	Y	Br	Y
α-*l*-Terpineol	PY	V	—	Gy	—	—
Geraniol	—	VBr	—	Gy	—	—
Nerol	—	VBr	—	Gy	—	—
Linalool	PBr	Y→V	—	YBr	—	—
Citronellol	—	V	—	Gy	—	—
Limonene	—	—	—	PYBr	—	—
α-Pinene	—	—	—	—	—	—
Menthol	—	—	—	—	—	—
Borneol	—	—	—	—	—	—

Br : Brown; G : Green; Gy : Gray; P : Pale; Or : Orange; R : Red; V : Violet; W : White; Y : Yellow; — : Colorless; → : Color changes
* Heated weakly after spraying.

Discussion and Conclusion

The writers' described method is an art of reversed-phase partition paper chromatography, but representation of Rf values is fairly lower than ordinary paper chromatography. This fact is thought to be due to the heterogeniety of filter paper and to treatment with paraffine and cetaceum. Therefore, experiments by this method requires comparative test of the sample.

Separation of terpenoid compounds on the materials used is worse in comparison with aromatic essential oils, for example eugenol, asarone, and safrole. As for the coloring reagents, Millon's and Denigè's solution are very sharp and the colors produced are variable. Millon's reagent cannot detect terpenoids, while Denigè's solution colors many terpenoides but scorches the paper. Menthol, pinene, and limonene have not good color reagent.

The writers hope to make further study on better developers and treatments of paper, and to find coloring reagents for terpenoids.

The writers express their deep gratitude to San'ei Chemical Ind. Co., Ltd., for the gift of many of the samples used.

Experimental

Preparation of essential oils—Isomethyleugenol was prepared by the methylation of isoeugenol. Asarone was separated from *Acorus gramineus* Soland.[5] by steam distillation.

Isoeugenol, pinene, and limonene were the commerical products. Eugenol, methyleugenol, safrole, nerol, geraniol, *l*-α-terpineol, linalool, citronellol, borneol, and menthol were supplied by San'ei Chemical Ind. Co., Ltd.

Summary

Paper chromatography of essential oils succeeded by the use of paraffine- and cetaceum-coated papers. Aromatic essential oils were well separated.

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5) Y. Kimura : J. Pharm. Soc. Japan, **46**, 380(1926).