Note

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Yohei Hashimoto and Jun-ichi Chatani : The Separation of Triterpenoids and their Related Compounds by Reversed-phase Chromatography.

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The method of reversed-phase paper chromatography by using silicone-treated paper was first applied by Tiselius and Kritchevsky[1] in the separation of several steroids and this method was immediately applied in the microidentification of essential oil components.[2]

The silicone-treated paper was prepared in much the same way as described in previous papers,[1,2] by using Toyo Roshi No. 52 and a benzene solution of Dow Corning Silicone No. 1107.

For the developing solvent, methanol, ethanol, propanol, or ethyl acetate is used and by these means, triterpenes and saponins can be successfully separated.

The chloroform solution of antimony trichloride was applied as a coloring reagent for the detection of steroids.[3,4] This was used in the microdetection of triterpenes in the present work, although filter paper changed dark in color because of heating procedure and thus spots were often difficult to distinguish from the background.

Therefore, the use of antimony pentachloride solution is preferable,[5] as it forms selective coloration by merely dipping the paper in the solvent without any heating. The unchanged white background made the spots more distinct. Several micrograms of triterpenes on paper can be detected, e.g. α-amyrin (15 γ), oleanolic acid (10 γ), ursolic acid (5 γ), and hederagenin (20 γ). The reagent of antimony pentachloride is generally applicable for the detection of triterpenes on filter paper.

Table I. Rf Value of Triterpenoids

Solvent No.	1	2	3	4	5	6
α-Amyrin	0.64	0.77	0.72	0.65	0.50	0.23
Ursolic acid	0.83	0.85	0.79	0.84	0.18	0.91
Uvaol	0.79	0.92	0.73	0.56	0.53	0.67
β-Amyrin	0.62	0.61	0.74	0.60	0.57	0.31
Hederagenin	0.77	0.96	0.71	0.71	0.0	0.79
Morolic acid	0.65	0.92	0.67	0.67	0.0	0.62
Oleanolic acid	0.75	0.88	0.82	0.76	0.08	0.57
Jegosapogenol	0.67	0.65	0.73	0.87	0.0	0.78
Lupeol	0.86	0.86	0.72	0.97	0.61	0.63
Sophoradiol	0.71	0.85	0.71	0.64	0.53	0.58
Sanguisorbigenin	0.74	0.87	0.75	0.69	0.41	0.54
Aokiol[a)]	0.73	0.77	0.83	0.74	0.0	0.78
Platycodigenin	0.66	0.82	0.87	0.79	0.0	0.61
Eburicoic acid	0.76	0.84	0.79	0.86	0.0	0.81
Squalene	0.0	0.0	0.85	0.65	0.65	0.92
Cholesterol	0.60	0.0	0.62	0.73	0.73	0.47

a) K. Kariyone, Y. Hashimoto, S. Osumi : Paper presented at the Kinki Local Meeting of the Pharmaceutical Society of Japan, Kyoto, November, 1951.

* Motoyama-cho, Higashinada-ku, Kobe (橋本庸平, 茶谷順一).

1) T.H. Kritchevsky, A. Tiselius : Science, **111**, 299(1951).
2) K. Hayashi, Y. Hashimoto : This Bulletin, **4**, 496(1956).
3) R. Neher, A. Wettstein : Helv. Chim. Acta, **35**, 276(1952).
4) Y. Hashimoto : Experientia, **9**, 194(1953).
5) C.R. Noller, R.A. Smith, G.H. Harris, T.W. Walker : J. Am. Chem. Soc., **64**, 3047(1942).

Table II. Rf Value of Saponins

Solvent No.	1	2	4	5	7	8
Saponin of *Polygala tenuifolia*	0.68	0.86	0.84	0.62	0.70	0.87
Jegosaponin	0.80	0.56	0.78	0.80	0.95	0.76
Sophorasaponin	0.59	0.86	0.64	0.52	0.65	—
Sanguisorbin	0.78	0.68	0.78	0.59	0.91	0.65
Quillajasaponin	0.75	0.83	0.57	0.44	0.70	0.73
Platycodin	0.82	0.81	0.68	0.68	0.67	0.74
Morasaponin	0.97	0.85	—	0.81	0.66	0.86
Glycyrrhizic acid	0.70	0.60	0.87	0.92	0.87	0.66

Solvent Composition
No. 1: 99% MeOH
2: EtOH:H_2O(1:1)
3: AcOEt
4: PrOH:toluene(1:1)
5: AcOH:H_2O(5:1)
6: Toluene:28% NH_4OH(10:1)(The supernatant layer was used)
7: Benzene:MeOH:H_2O(10:6:1)
8: AcOEt:10% MeOH(5:1)

Table III. Color Reaction of Sapogenins and Saponins by $SbCl_3$ and $SbCl_5$

	$SbCl_5$	$SbCl_3$		$SbCl_5$	$SbCl_3$
α-Amyrin	YBr — DV	R	Betulin	Y	R
Ursolic acid	YBr — V	RP	Taraxasterol	Y — DV	R
Uvaol	Or — V	Or	Sophoradiol	DBr	R
β-Amyrin	Br — DR	R	Sanguisorbigenin	Br — V	R
Hederagenin	Y — V	R	Aokiol	Y — Br	Y
Morolic acid	Y — R	R	Platycodigenin	Or — Br	Or
Oleanolic acid	Y — R	R	Squalene	DBr	Br
Jegosapogenol	Br — R	R	Cholesterol	YBr — DBr	R
Lupeol	Y	Br	Quillajasaponin	R	R
Jegosaponin	Br — R	R	Morasaponin	R — V	R
Sanguisorbin	Br — V	R	Sophorasaponin	R	Br

Br: Brown Or: Orange R: Red V: Violet Y: Yellow
P: Pale D: Deep — Color changes

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Experimental

Preparation of Silicone-treated Paper Strips—The strips (1×25 cm.) of filter paper, Toyo Roshi No. 52, were immersed in 5% benzene solution of Dow Corning Silicone No. 1107. After removing excess solution by pressing between several sheets of dry filter paper, benzene was evaporated, and strips were heated in an electric oven up to 120° for 10 mins.

Coloring Reagent—The finished papergrams were immersed in 10% $SbCl_3$ or 20% $SbCl_5$ solution and dried. In the case of former reagent, paper strips were heated to 100° during 2 mins., while color reaction of the latter was readily revealed without any treatment.

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