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Note

Substitution of methyl *tert.*-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products

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A standardized thin-layer chromatographic (TLC) method[1–8] for the identification of lichen products uses three solvent systems, and spots are initially sorted by R_F classes defined on each chromatogram by internal control substances (atranorin and norstictic acid). One of the three solvent systems (solvent B) contains hexane, diethyl ether and formic acid. Lichenologists surveying large numbers of specimens in chemosystematic studies have found this solvent difficult to use because the diethyl ether component of the solution rapidly evaporates. In addition, recent concern over the hazard in the use and storage of diethyl ether has led some workers to discontinue the use of solvent B and to rely upon only solvents A and C. Solvents A and C, however, are much less useful than solvent B for the separation of closely related compounds differing primarily by hydrophobicity. A higher-boiling ether, methyl *tert.*-butyl ether (MTBE), has been shown[9] to be a good substitute for diethyl ether in ether–hexane solvent systems for TLC and normal phase high-performance liquid chromatographic (HPLC) separations of salicylic acid and a variety of monoaromatic phenols that are somewhat similar to the phenolic acid precursors of several categories of di- and triaromatic lichen products. The present note reports a solvent system that contains MTBE instead of diethyl ether and that has chromatographic properties nearly identical to those of solvent B of the standardized method for lichen products.

EXPERIMENTAL

Chromatograms were prepared as described elsewhere[1–3,6] on Merck silica gel 60 F_{254} (0.25-mm layer; No. 5765) plates shortened to 12.5 cm and run to a height of 10 cm from the origin (2 cm from the bottom of the plate). Controls of atranorin and norstictic acid were included at three positions on every plate. Spotted plates were pre-equilibrated 5 min over 60% formic acid[3]. MTBE was HPLC grade (E-127; Fisher Scientific, Pittsburg, PA, U.S.A.) but the product classified as Purified (E-129) gave very similar chromatographic results. A hexane–MTBE–formic acid (140:72:18) solvent gave chromatograms most similar to those obtained with solvent B (hexane–diethyl ether–formic acid; 120:90:20). Chromatograms were air-dried (hood), viewed under short- and longwave UV light and visualized with a spray of 10% sulfuric acid and heat (15–30 min at 110°C). $R_F \times 100$ values were 10–15% higher than normal on the first one or two chromatograms and then decreased only very slowly on sub-

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sequent plates. The level of solution in the tank was maintained near 230 ml with additions of fresh solvent. The $R_F \times 100$ value of norstictic acid was near 30, but values in the range of 26–38 still permitted accurate determinations of R_F classes.

MTBE was compared to diethyl ether as the solvent used for the extraction step in the hydrolysis of depsides[2] using perlatolic acid and barbatic acid to test the procedure. Although the hydrolysis products appeared to dissolve less rapidly in MTBE, this solvent gave satisfactory results in both cases.

RESULTS AND DISCUSSION

Standardized R_F data for solvent B (MTBE) (Table I) determined for examples of all major categories of lichen products (*e.g.*, orcinol and β-orcinol depsides and depsidones, dibenzofurans, xanthones, anthraquinones, pulvinic acids, fatty acids and triterpenes) corresponded closely to the values recently found in our laboratory for solvent B. The useful lifetime of either solvent depends upon the number of chromatograms developed and the time that the solvent is stored in the developing tank. Compared to solvent B, the new solvent system gives satisfactory results for more successive chromatograms and can be left in a closed chromatographic tank (preferably the type with a flanged edge and lid) for a longer time before the R_F values of the atranorin and norstictic acid controls drop below acceptable limits.

The higher boiling and flash points of MTBE (55.2 and −28°C, respectively) compared to diethyl ether (34.5 and −45°C) and the lower tendency to form peroxides greatly reduce fire and explosion hazard. If MTBE is substituted for diethyl ether in solvent B and also used as the extraction solvent in the procedure for the hydrolysis of lichen depsides, there is no need to stock diethyl ether for routine analyses of lichens. This is a special advantage to lichenologists, many of whom work in museums and herbaria that are not equipped to store hazardous materials. We recommend the substitution of MTBE for diethyl ether in solvent B and as the extraction solvent for hydrolysis experiments in chemosystematic studies of lichen-forming fungi.

TABLE I

$R_F \times 100$ VALUES STANDARDIZED AGAINST NORSTICTIC ACID AND ATRANORIN FOR 115 LICHEN PRODUCTS IN SOLVENT B (MTBE)

Compound	R_F *class*	R_F/R_FN, R_FA[a] *(all values × 100)*
Consalazinic	1	1/33, 77
Constictic	1	1.5/32, 74
Erythrin[b]	2	4/33, 74
Salazinic	2	7/32, 73
PQ-4[c]	2	7/32, 77
Stictic	2	9/32, 73
Cryptostictic	2	10/32, 73
Lepraric[d]	2	11/33, 74
Connorstictic	2	11/32, 73
Variolaric[b]	2	11/33, 77

TABLE I *(continued)*

Compound	R_F *class*	R_F/R_FN, R_FA^a *(all values × 100)*
Porphyrilic	2	12/33, 77
Secalonic A[b,d]	1–2	13/31, 74
Menegazziaic[b]	2–3	15/32, 73
Aspicilin[b]	2–3	17/33, 73
Galbinic[b]	2–3	17/32, 73
Decarboxythamnolic[b]	2–3	18/33, 75
Succinprotocetraric	3	18/31, 73
Protocetraric	3	19/31, 73
Unknown SV-1[d,e]	2–3	20/33, 73
Thamnolic[b]	3	21/33, 75
Strepsilin	3	22/29, 73
Squamatic	3	25/33, 74
Schizopeltic[b]	3	25/32, 74
Hyposalazinic	3	26/32, 77
Fumarprotocetraric	3	26/31, 73
Convirensic[f]	3	28/33, 74
Echinocarpic[b]	4	31.5/33, 75
Endocrocin	4	31.5/32, 74
Alectoronic	4	31/31, 72
Caperatic	4	32/32, 72
Hypostictic	4	32/32, 80
4′-O-Methylpaludosic	4	34/32, 71
Physodalic[b]	4, 5	33/31, 73
4-O-Demethylnotatic	5	33/29, 73
α-Collatolic	5	35/31, 72
Confluentic	5	36/32, 72
Skyrin	5	36/32, 74
Norrangiformic	5	36/32, 73
Diploschistesic	5	37/33, 74
Lividic	5	37/32, 73
Norlobaridone	5	36/30, 72
2′-O-Demethylpsoromic	5	39/33, 74
2,4-Di-O-methylgyrophoric	5	35/28, 72
Physodic	5	35/28, 72
Gangaleoidin[b]	5	38/30, 72
Unknown E-1[g]	5	40/33, 75
Olivetoric	5	41/33, 73
Gyrophoric	5	42/33, 73
Rangiformic	5	41/32, 73
Hypoprotocetraric	5	37/29, 73
2-O-Methylsekikaic	5	40/32, 71
Planaic	5	40/32, 73
Paludosic	5	42/32, 71
Baeomycesic	5	41/33, 74
Microphyllinic	5	42/33, 74
Rhizocarpic	5	42/31, 73
Lecanoric	5	44/33, 73
Methyl gyrophorate	5	39/28, 72
Cryptochlorophaeic	5	45/32, 71
(−)-*allo*-Protolichesterinic	5	43/32, 76

(Continued on p. 486)

TABLE I *(continued)*

Compound	R_F *class*	R_F/R_FN, R_FA^a *(all values × 100)*
Boninic	5	44/32, 71
Picrolichenic	5	45/33, 73
Notatic	5	46/33, 74
Psoromic	5	46/33, 74
4-O-Methylphysodic	5	45/31, 73
Zeorin	5	47/33, 73
Orsellinic	5	47/33, 72
Lobaric	5	47/32, 73
4,4'-Di-O-methylcryptochlorophaeic	5	47/32, 71
Methyl lecanorate	5	48/33, 72
Unknown Pmc-1[b,i]	5	48/33, 74
Epanorin	5	47/31, 73
4'-O-Methylnorhomosekikaic	5	48/32, 71
Norobtusatic	5	48/31, 74
Protolichesterinic	5	46/32, 77
4-O-Methylgyrophoric	5	50/31, 73
Ursolic	5	48/28, 72
Alectorialic	5	50/32, 73
Merochlorophaeic[b]	5–6	52/32, 71
Unknown CS-1[j]	5–6	52/32, 72
4-O-Demethylbarbatic	5–6	52/31, 74
Thiophanic	5–6	52/31, 73
4-O-Methylhypoprotocetraric	5–6	53/33, 74
Scrobiculin	5–6	55/32, 74
Tenuiorin	5–6	55/33, 72
Anziaic	5–6	55/33, 73
Diffractaic	5–6	55/31, 74
Evernic	5–6	53/28, 73
Lichesterinic	6	58/32, 77
Virensic[b]	6	56/33, 74
4-O-Methylcryptochlorophaeic[b]	6	56/32, 71
Unknown Lgn-1[k]	6	56/30, 72
Emodin	6	58/32, 74
Sekikaic[b]	6	57/32, 71
Grayanic[b]	6	59/33, 74
Vulpinic	6	61/31, 73
Obtusatic	6	63/31, 74
Bourgeanic[b]	6	62/32, 72
Homosekikaic[b]	6	62/32, 71
Pannarin	6	64/33, 74
Diploicin	6	65/30, 72
Lichexanthone	6	66/31, 73
Barbatic	6	67/31, 74
Usnic[d]	6	65/32, 71
Divaricatic	6	65/32, 73
Colensoinic	6	68/32, 73
Parietin	6	69/32, 74
Vicanicin	6	68/30, 72
Didymic[b]	6–7	68/28, 72
Imbricaric	6–7	69/30, 72
Stenosporic	6–7	70/30, 72
Sphaerophorin	7	71/30, 72

TABLE I *(continued)*

Compound	R_F *class*	R_F/R_FN, R_FA[a] *(all values × 100)*
Perlatolic	7	73/30, 72
Methyl barbatate	7	75/32, 74
Calycin	7–8	79/32, 76

[a] The two numbers following the oblique (/) are measurements in millimeters to the norstictic acid line (R_FN) and the atranorin line (R_FA)[1].

[b] R_F relative to standards somewhat lower than with solvent B.

[c] In *Xanthoparmelia quintaria* (Hale) Hale.

[d] A trailing spot difficult to measure.

[e] A common accessory pigment in lichens.

[f] In *Sulcaria virens* (Tayl.) Bystr. *ex* Brodo & Hawksw.; colors yellow with 10% sulfuric acid visualization.

[g] An unknown substance in species with echinocarpic acid.

[h] R_F relative to standards somewhat higher than with solvent B.

[i] An unknown substance in many species with virensic acid (*e.g.*, *Sulcaria virens*) and/or protocetraric acid [*e.g.*, *Parmotrema michauxianum* (Zahlbr.) Hale]; colors gray with 10% sulfuric acid visualization.

[j] An aliphatic unknown in *Cladonia subcariosa* Nyl.

[k] With gangaleoidin in *Lecanora gangaleoides* Nyl.; not the unknown with gangaleoidin in *L. californica* Tuck. and *L. meridionalis* Magn.

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