ACTA CHEMICA SCANDINAVICA 21 (1967) 1162–1172

Chemical Studies on Lichens

4.* Thin Layer Chromatography of Lichen Substances

JOHAN SANTESSON

The Institute of Chemistry, University of Uppsala, Uppsala, Sweden

The thin layer chromatography on precoated plates of more than eighty lichen substances is described.

Several authors have studied the thin layer chromatography of lichen substances. Ramaut separated depsides [1] and β-orcinol depsidones,[2] as did Bachmann.[3] Pulvic acid derivatives were chromatographed by Bendz *et al.*[4] and Harper and Letcher.[5] The aldehydic depsides and β-orcinol depsidones were studied by Santesson [6] and the aliphatic lichen acids by Bendz *et al.*[7] In all these cases hand-coated silica gel plates were used for the separations.

Since precoated plates offer a quicker and more convenient way of chromatographically identifying organic compounds, the separation of lichen substances has been studied, using such plates. The results of this investigation are presented in this paper.

Lichen substances can be grouped according to either their biosynthesis,[8] their main structural features,[9] or the colour reactions obtained by certain widely used reagents.[10] For chromatographical purposes, a combination of the two latter techniques seems to be most useful. Consequently, in this paper, the lichen substances discussed are grouped as follows:

1. Aldehydic, aromatic compounds, giving a positive colour reaction with *p*-phenylenediamine [11] or *o*-dianisidine [6,12] (Table 1).

2. Depsides and chromenones with two free hydroxyl groups in the *meta* position, giving a red colour reaction with calcium hypochlorite.[13] Diploschistesic acid is also included in this group, although it produces a bluish violet colour instead of a red one (Table 2, "C + red, PD—" compounds).

3. Depsides, giving a red colour with potassium hydroxide, but no colour as in 1 or 2 (Table 3, "K + red, PD—, C—" compounds).

4. Depsides, depsidones, and depsones, not belonging to any of the preceding groups, giving a positive reaction (red colour) with calcium hypochlorite

* Part 3: *Phytochemistry* 6 (1967) 685.

Table 1. Aromatic aldehydes.

Compound	$R_F \times 100$ in solvent system				Source for the compound
	A	B	C	D	
Atranorin	82	94	85	60	*Parmelia physodes* (L.) Ach.
Baeomycesic acid	52	83	71	*a*	*Thamnolia subuliformis* (Ehrh.) W. Culb.
Barbatolic acid	51	60	36	*a*	*Alectoria implexa* (Hoffm.) Nyl.
Chloroatranorin	81	94	85	39	*Parmelia physodes* (L.) Ach.
Fumarprotocetraric acid	10	46	25	*a*	*Cetraria islandica* (L.) Ach.
Norstictic acid	34	81	66	*a*	*Parmelia acetabulum* (Neck.) Duby
Pannarin	81	93	85	49	*Pannaria fulvescens* (Mont.) Nyl.
Physodalic acid	34	62	46	*a*	*Parmelia physodes* (L.) Ach.
Protocetraric acid	10	52	33	*a*	*Ramalina farinacea* (L.) Ach.
Psoromic acid	52	82	73	*a*	C.A.W. (*Rhizocarpon geographicum* (L.) DC)
Salazinic acid	06	49	29	*a*	*Parmelia saxatilis* (L.) Ach.
Stictic acid	21	51	26	*a*	*Parmelia conspersa* (Ehrh.) Ach.
Thamnolic acid	21	45	22	*a*	*Thamnolia vermicularis* (Sw.) Ach.

[a] $R_F \times 100$ does not exceed 10.

A: Toluene-glacial acetic acid, 9:1 (v/v).
B: Toluene-diethyl ether-glacial acetic acid, 3:6:1 (v/v/v).
C: Toluene-diethyl ether-glacial acetic acid, 7:12:1 (v/v/v).
D: Cyclohexane-chloroform-methyl ethyl ketone, 30:15:2 (v/v/v).

Table 2. *m*-Dihydroxy-phenols.

Compound	$R_F \times 100$ in solvent system			Colour with Echtblau-salz B	Source for the compound
	A	B	C		
Anziaic acid	79	70	03	red violet	*Anzia opuntiella* Müll. Arg.
Diploschistesic acid	59	30	01	brown yellow	*Diploschistes scruposus* (Schreb.) Norm.
Erythrin	09	04	01	violet	*Schizopelte californica* Th. Fr.
Gyrophoric acid	64	45	01	red violet	*Umbilicaria spodochroa* (Ach.) Hoffm.
Hiascic acid [a]	40	23	01	brown yellow	*Cetraria delisei* (Bory) Th. Fr.
Hypothamnolic acid	07	71	02	yellow brown	*Siphula roccellaeformis* Nyl.
Lecanoric acid	70	45	01	red violet	*Parmelia fuliginosa* (Duby) Nyl.
Methyl 3,5-dichlorolecanorate	86	90	59	brown	*Ramalina flaccescens* Nyl.
Olivetoric acid	70	63	02	red violet	*Parmelia furfuracea* (L.) Ach. var. *olivetorina* (Zopf) Zahlbr.
Siphulin	47	10	01	violet	*Siphula ceratites* (Fr.) Th. Fr.

[a] Accessory compound to gyrophoric acid.

A: Diethyl ether-glacial acetic acid, 50:1 (v/v).
B: Dichloromethane-glacial acetic acid, 9:1 (v/v).
C: Dichloromethane.

Table 3. "K+ red" depsides.

Compound	$R_F \times 100$ in solvent system		Colour with Echtblausalz B	Source for the compound
	A	B		
Cryptochlorophaeic acid	45	65	brown	C.C. *(Parmelia cryptochlorophaea* Hale)
Gyrophoric acid[a]	26	45	red violet	*Umbilicaria spodochroa* (Ach.) Hoffm.
Hiascic acid	08	23	red brown	*Cetraria delisei* (Bory) Th.Fr.
Ramalinolic acid	33	56	red violet	*Ramalina usnea* (L.) Howe
Sekikaic acid[b]	56	81	red brown	S.H.; *Ramalina usnea* (L.) Howe

[a] Accessory compound to hiascic acid. [b] Accessory compound to ramalinolic acid.

A: Toluene-glacial acetic acid, 9:1 (v/v). B: Dichloromethane-glacial acetic acid, 9:1 (v/v).

Table 4. "KC+, PD−, C−, K−" compounds.

Compound	$R_F \times 100$ in solvent system				Colour with *bis*-diazotized benzidine	Source for the compound
	A	B	C	D		
Alectoronic acid	06	10	10	14	R	*Parmelia centrifuga* (L.) Ach.
α-Collatolic acid	24	29	29	50	R	C.A.W. *(Lecanora atra* (Hudson) Ach.)
Glomelliferic acid	34	36	37	50	Y−R	*Parmelia glomellifera* Nyl.
Lobaric acid	28	31	32	42	Y−R	*Stereocaulon pascale* (L.) Hoffm.
4-*O*-Methylphysodic acid	11	18	21	34	Y	*Parmelia livida* Tayl.
Microphillinic acid	30	35	33	43	R	*Cetraria japonica* Zahlbr.
Obtusatic acid	37	43	53	50	Y	*Ramalina pollinaria* Ach.
Physodic acid	04	06	05	09	R	*Parmelia physodes* (L.) Ach.
Picrolichenic acid	32	34	40	39	R	C.A.W. *(Pertusaria amara* (Ach.) Nyl.)

A: Toluene-glacial acetic acid, 25:1 (v/v). B: Toluene-glacial acetic acid, 9:1 (v/v).
C: Toluene-formic acid, 9:1 (v/v). D: Toluene-hexane-glacial acetic acid, 3:3:1 (v/v/v).

Colour legend: R = red-brown, Y = yellow-yellow brown. Only the principle colour obtained with *bis*-diazotized benzidine is given, since there are often slight variations.

Table 5. Dibenzofuranes.

Compound	$R_F \times 100$ in solvent system				Source for the compound
	A	B	C	D	
Didymic acid	80	81	90	79	*Cladonia didyma* (Fée) Vain.
Pannaric acid	36	35	36	20	C.A.W. *(Crocynea membranacea* (Dicks.) Zahlbr.)
Porphyrilic acid	44	40	54	39	C.A.W. *(Haematomma coccineum* (Dicks.) Körb. v. *coccineum)*
Schizopeltic acid (*)	80	83	95	85	*Schizopelte californica* Th. Fr.; *Reinkella parishii* Hasse
Strepsilin	69	56	73	65	*Cladonia strepsilis* (Ach.) Vain.

A: Toluene-acetone-glacial acetic acid, 7:2:1 (v/v/v).
B: Carbon tetrachloride-methyl ethyl ketone-glacial acetic acid, 6:2:1 (v/v/v).
C: Dichloromethane-acetone-glacial acetic acid, 6:1:1 (v/v/v).
D: 1,1,2,2-Tetrachloroethane-acetone-glacial acetic acid, 8:1:1 (v/v/v).

Table 6. Quinonoid pigments.

Compound	$R_F \times 100$ in solvent system			Colour of the spot	Source for the compound
	A	B	C		
Emodin	03	04	*a*	yellow orange	commercial
Endocrocin	00	00	45	yellow	*Nephromopsis endocrocea* Asahina
Fragilin	54	63	*a*	yellow	*Sphaerophorus fragilis* (L.) Pers.
Lauropurpone (*)	38	51	*a*	orange red	C.A.W. *(Laurera purpurina* (Nyl.) Zahlbr.)
Mysaquinone (*)	00	00	08	violet red	*Mycoblastus sanguinarius* (L.) Norm.
Parietin	53	59	*a*	yellow	*Xanthoria parietina* (L.) Th. Fr.
Parietinic acid	00	00	65	yellow	synthetic
Polyporic acid	73	85	*a*	yellow	*Sticta colensoi* Bab.
Rhodocladonic acid	00	00	05	red violet	*Cladonia* sp.
Solorinic acid	57	74	*a*	orange yellow	*Solorina crocea* (L.) Ach.

[a] $R_F \times 100$ values exceed 90. A: Toluene-cyclohexane, 4:1 (v/v). B: Toluene. C: Ethanol (95 %).

Table 7. Pulvic acid, its derivatives and usnic acid.

Compound	$R_F \times 100$ in solvent system				Colour of the spot in		Source for the compound
	A	B	C	D	daylight	UV (365 mμ)	
Calycin	73	60	81	56	orange red	dark brown red	*Chrysotrix noli tangere* (Mont.) Mont.
Epanorin	83	80	84	57	yellow	orange	synthetic
Pinastric acid	58	40	86	19	yellow	orange	*Cetraria juniperina* (L.) Ach.
Pulvic acid	00	00	27	00	yellow	yellow	synthetic
Pulvic dilactone	90	83	92	75	yellow	yellow	synthetic
Rhizocarpic acid	82	78	84	53	yellow	orange	*Acarospora chlorophana* (Wg) Mass.
Usnic acid	78	62	82	51	faint yellow	dark	*Usnea magellanica* (Mont.) Mot.
Vulpinic acid	47	29	85	17	yellow	yellow	synthetic

A: Chloroform-acetone, 4:1 (v/v). B: Hexane-chloroform-acetone, 2:3:1 (v/v/v).
C: Toluene-glacial acetic acid, 4:1 (v/v). D: Hexane-chloroform-acetone, 10:8:1 (v/v/v).

Table 8. Xanthones and chromones.

Compound	$R_F \times 100$ in solvent system				Colour in UV (365 mμ) after exposure to ammonia vapour	Source for the compound
	A	B	C	D		
Deschlorothiophanic acid (*)	33	16	00	22	orange	*Lecanora straminea* Ach.
Flavicanone (*)	56	69	08	40	orange	C.A.W. *(Pertusaria flavicans* Lamy)
Lichexanthone	74	69	71	86	orange	synthetic
Rupicolin (*)	56	54	36	76	dark	S.H. *(Lecanora rupicola* (L.) Zahlbr.)
Thuringion (*)	52	58	20	66	yellow	S.H. *(Lecidea carpathica* (Körb.) Szat.)

A: Toluene-glacial acetic acid, 9:1 (v/v). B: Toluene-butyric acid 9:1, (v/v.)
C: Chloroform (stabilized with 1 % ethanol). D: Dichloromethane-acetone, 4:1 (v/v).

Table 9. Various depsides and depsidones.

Compound	$R_F \times 100$ in solvent system					Colour with *bis*-diazotized benzidine	Source for the compound
	A	B	C	D	E		
Barbatic acid	40	54	34	*a*	*a*	R	*Cladonia floerkeana* (Fr.) Sommerf.
Boninic acid	22	42	35	51	*a*	Y	*Ramalina boninensis* Asahina
Confluentinic acid	24	43	28	*a*	*a*	R	S.H.
Diffractaic acid	38	53	32	*a*	*a*	R	*Usnea diffracta* Vain.
Divaricatic acid	32	49	27	*a*	*a*	R	*Evernia divaricata* (L.) Ach.
Evernic acid	27	45	20	*a*	*a*	R	*Evernia prunastri* (L.) Ach.
Grayanic acid	20	38	24	*a*	*a*	Y	C.C. *(Cladonia grayi* Merr.)
Homosekikaic acid	46	67	45	*a*	*a*	Y	*Cladonia nemoxyna* (Ach.) Coëm.
Hypothamnolic acid[b]	03	06	01	25	34	Y	*Siphula roccellaeformis* Nyl.
Sekikaic acid	43	56	37	*a*	*a*	R	S.H.; *Ramalina usnea* (L.) Howe
Sphaerophorin	43	60	38	*a*	*a*	R	*Sphaerophorus fragilis* (L.) Pers.
Squamatic acid	05	07	03	30	56	Y	*Thamnolia subuliformis* (Ehrh.) W. Culb.
Variolaric acid	02	04	02	21	42	R	*Pertusaria lactea* (L.) Arn.
secondary front	58	70	55	57	82	—	—

[a] Travels with the secondary front.

[b] Since the momentary red colour that hypothamnolic acid gives with calcium hypochlorite often is very hard to observe, this acid is also included here.

Colour legend: R = red-brown, Y = yellow-yellow brown. Only the principle colour obtained with *bis*-diazotized benzidine is given, since there are often slight variations.

A: Toluene-butyric acid, 19:1 (v/v).
B: Toluene-butyric acid, 9:1 (v/v).
C: Dichloromethane-hexane-butyric acid, 10:9:1 (v/v/v).
D: Toluene-glacial acetic acid, 9:1 (v/v).
E: Dichloromethane-glacial acetic acid, 9:1 (v/v).

Table 10. Aliphatic lichen acids.

Compound	$R_F \times 100$ in solvent system		Source for the compound
	A	B	
Acaranoic acid (*)	60	*a*	*Acarospora chlorophana* (Wg) Mass.
Acarenoic acid (*)	48	*a*	*Acarospora chlorophana* (Wg) Mass.
Caperatic acid	08	28	L.T. (*Parmelia caperata* (L.) Ach.)
Lichesterinic acid	62	*a*	L.T. (synthetic)
Nephromopsinic acid	70	*a*	L.T. (*Nephromopsis stracheyi* (Bab.) Müll. Arg. f. *ectocarpisma* Hue)
Norrangiformic acid	10	36	L.T. (synthetic)
Protolichesterinic acid	66	*a*	*Cetraria islandica* (L.) Ach.
Rangiformic acid	61	*a*	L.T. (*Cladonia rangiformis* Hoffm.)
Roccellic acid	75	*a*	*Roccella phycopsis* (Ach.) Ach.

[a] Travels with the secondary front ($R_F \times 100 = 82$).

A: Diethyl ether-butyric acid, 30:1 (v/v).
B: Dichloromethane-glacial acetic acid, 9:1 (v/v).

after previous treatment with potassium hydroxide (Table 4, "KC +, PD—, C—, K—" compounds).

5. Dibenzofuranes, giving a green-blue colour reaction with calcium hypochlorite (with one exception) (Table 5, "C+ green-blue, PD—, K—" compounds).

6. Quinones (yellow-violet coloured pigments), giving a red-violet black colour with potassium hydroxide (Table 6, "K+" pigments).

7. Pulvic acid, its derivatives, and usnic acid, — yellow pigments, which, with the exception of usnic acid, do not give any characteristic colour reactions (Table 7).

8. Xanthones and chromones (cream-coloured — yellow compounds) which often give a deep yellow colour with calcium hypochlorite (Table 8).

9. Various depsides and depsidones, not belonging to any of the preceding groups, which react with *bis*-diazotized benzidine (Table 9, "PD—, C—, K—, KC—" compounds).

10. Aliphatic lichen acids, which do not give any characteristic colour reactions (Table 10).

Two main groups of lichen substances, sugar alcohols, and terpenes, and two small groups, sulphur-containing compounds and diketo-piperazine derivatives, are not treated in this paper.

The presence or absence of compounds, belonging to the groups 1—6, in a lichen specimen is usually ascertained by carrying out the respective colour reactions directly upon the lichen thallus. In many cases, the changes are difficult to observe. A more sensitive method, described in the experimental part, is "the filter paper method", in which an extraction of a small lichen fragment is carried out upon a filter paper.

The results of the present investigation are summarized in Tables 1—10. Some lichen substances, which frequently occur together with compounds belonging to other groups, will be found not only in the appropriate tables, but also in the tables in which the accessory compounds appear. A few compounds, whose structures have not yet been published, are also included in the tables. These are denoted by an asterisk within parentheses (*) and the names of the compounds must be regarded as tentative. Emodin, included in Table 6, has not yet been found in lichens, but in view of the occurrence of parietin, 6-*O*-methyl-emodin, in numerous lichens, it was considered desirable to include it.

The R_F values may vary somewhat from one plate to another, but the sequence of the substances is always the same. It must be stressed, however, that no compound should be identified by the R_F value alone. Co-chromatography in more than one solvent system should always be carried out, if identification is sought.

EXPERIMENTAL

Chromatography. The thin layer chromatography was carried out on Eastman "Chromagram" sheets, type K 301 R 2, cut down to a height of 6.7 cm. The plates were activated at 105°C for 15 min and stored over silica gel. The spots were applied 1.0 cm above the lower edge and the solvent was allowed to travel to a line 0.7 cm from the upper edge. Care was taken, not to let the applied spots exceed 0.1 cm in diameter. The plates were dried before spraying with the appropriate reagent.

The solvents used were of *puriss.* or *purum* quality. In the latter case, they were purified by distillation and/or passing through a column of neutral alumina, activity grade I.

Detection of the spots. The spots were made visible by the following methods. Compounds listed in Table 1 (aromatic aldehydes) gave yellow-red Schiff bases when sprayed with an 0.1 % ethanolic solution of *o*-dianisidine.[6] The depsides and the chromenones of Table 2 ("C+ red" compounds) and Table 3 ("K+ red, PD−, C−" compounds) gave yellowish-brown colours if sprayed first with an 0.1 % aqueous solution of Echtblausalz B, then with a 1.0 % potassium hydroxide solution and finally heated to 100°C for 30−45 sec.[14]

By means of *bis*-diazotized benzidine as described by Lindstedt [15] (*cf.* Refs. 14,16), the compounds listed in Table 4 ("KC+, PD−, C−, K−" compounds) and Table 9 ("PD−, C−, K−, KC−" compounds) were easily made visible. In the case of picrolichenic acid (Table 4) the plates were exposed to ammonia vapour for 1 min (in order to hydrolyse the depsone [17]) prior to spraying. The dibenzofuranes (Table 5) could be detected either by their intense bluish white fluorescence in UV light (365 mμ) after exposure (1 min) to ammonia vapour or by the green-blue colour produced by spraying the plates with a dilute (0.5 %) solution of calcium hypochlorite. In the latter case, schizopeltic acid does not react.

The quinones (Table 6, "K+" pigments) were easily seen without the use of any reagent. Pulvic acid, its derivatives, and usnic acid (Table 7) were best detected in UV light (365 mμ) without previous treatment of the plates. Xanthones and chromones (Table 8) were detected in the same way, but after exposure to ammonia vapour. The aliphatic acids (Table 10) were made visible by spraying the plates with a solution of 40 mg bromocresol green in 100 ml 0.01 N sodium hydroxide.[7]

Reference compounds. Most reference compounds were isolated and purified according to Asahina and Shibata [18] from the respective lichens mentioned in the tables. Some compounds were obtained as gifts from Dr. Chicita Culberson, (quoted as C.C. in the tables), Dr. Siegfried Huneck (S.H.), fil. kand. Leif Tibell (L.T.) and Dr. Carl Axel Wachtmeister (C.A.W.). Methyl 3,5-dichlorolecanorate was isolated according to Bendz *et al.*,[19] siphulin as described by Bruun,[20] fragilin according to Bruun *et al.*,[21] whereas emodin was commercially available. Parietinic acid was synthetized from parietin according to Eder and Hauser.[22] Pulvic acid, pulvic dilactone, and vulpinic acid were synthetised by the method of Volhard,[23] epanorin by the method of Frank *et al.*,[24] hypoprotocetraric acid by reduction of fumarprotocetraric acid as described by Culberson,[25] and lichexanthone by the method of Grover *et al.*[26]

In the case of substance with unpublished structures, the isolation methods will be reported upon: schizopeltic acid,[27,28] mysaquinone,[28] lauropurpone,[29] deschlorothiophanic acid,[27,28] rupicolin,[30] flavicanone,[31] acaranoic acid and acarenoic acid.[28]

4-*O*-Methylphysodic acid [32] was not isolated, but an unpurified extract of *Parmelia livida* was used.

"The filter paper method". For a preliminary examination of lichen specimens to be investigated by thin layer chromatography, the following method was found convenient. A small piece of the lichen specimen is pressed down in the middle of a filter paper. By dropwise treatment with acetone (10−20 drops) the lichen substances present are extracted. Each drop of acetone is allowed to evaporate, leaving the extracted substances in a ring around the lichen fragment. This fragment is removed and tests with the appropriate colour reagents are carried out upon the "extract ring". Any colour reactions are far more easily observed than if the same tests were made upon the lichen thallus.

Acknowledgements. The author wishes to express his sincere thanks to the head of the institute, Professor Arne Fredga, for the facilities put at his disposal, to Dr. Gerd Bendz for her kind interest in this study, to Dr. Chicita Culberson, Dr. Siegfried Huneck, fil.kand. Leif Tibell, and Dr. Carl Axel Wachtmeister for reference compounds, and to Dr. Rolf Santesson for the supply and identification of almost all of the lichens used, without whose help this investigation would not have been possible. A grant from the *Faculty of Mathematics and Natural Sciences* at the University of Uppsala is gratefully acknowledged.

REFERENCES

1. Ramaut, J. L. *Bull. Soc. Chim. Belges* **72** (1963) 316.
2. Ramaut, J. L. *Bull. Soc. Chim. Belges* **72** (1963) 97.
3. Bachmann, O. *Oesterr. Botan. Z.* **110** (1963) 103.
4. Bendz, G., Santesson, J. and Wachtmeister, C. A. *Acta Chem. Scand.* **19** (1965) 1776.
5. Harper, S. H. and Letcher, R. M. *Proceedings and Transactions of the Rhodesia Scientific Association* **51** (1966) 156.
6. Santesson, J. *Acta Chem. Scand.* **19** (1965) 2254.
7. Bendz, G., Santesson, J. and Tibell, L. *Acta Chem. Scand.* **20** (1966) 1181.
8. Shibata, S. *Beitr. Biochem. Physiol. Naturstoffen, Festschr.* **1965** 451.
9. Shibata, S. In Linskens, H. F. *Modern Methods of Plant Analysis*, Vol. VI, Berlin 1963.
10. Hale, M. E., Jr. *Lichen Handbook*, Washington D. C. 1961, pp. 69—71.
11. Asahina, Y. *Acta Phytochim.* **8** (1934) 47.
12. Santesson, J. *Lichenologist* **3** (1966) 215.
13. Zopf, W. *Die Flechtenstoffe*, Jena 1907.
14. Hess, D. *Planta* **52** (1958) 65.
15. Lindstedt, G. *Acta Chem. Scand.* **4** (1950) 448.
16. Wachtmeister, C. A. *Botan. Notiser* **109** (1956) 313.
17. Wachtmeister, C. A. *Acta Chem. Scand.* **12** (1958) 147.
18. Asahina, Y. and Shibata, S. *Chemistry of Lichen Substances*, Tokyo 1954.
19. Bendz, G., Santesson, J. and Wachtmeister, C. A. *Acta Chem. Scand.* **19** (1965) 1188.
20. Bruun, T. *Acta Chem. Scand.* **19** (1965) 1677.
21. Bruun, T., Hollis, D. P. and Ryhage, R. *Acta Chem. Scand.* **19** (1965) 839.
22. Eder, R. and Hauser, F. *Helv. Chim. Acta* **8** (1925) 126.
23. Volhard, J. *Ann.* **282** (1894) 1.
24. Frank, R. L., Cohen, S. K. M. and Coker, J. N. *J. Am. Chem. Soc.* **72** (1950) 4454.
25. Culberson, C. *Phytochemistry* **4** (1965) 951.
26. Grover, P. K., Shah, G. D. and Shah, R. C. *J. Sci. Ind. Res. (India)* **B 15** (1956) 629.
27. Huneck, S. *To be published.*
28. Santesson, J. *To be published.*
29. Stensiö, K.-E. and Wachtmeister, C. A. *To be published.*
30. Huneck, S. and Santesson, J. *To be published.*
31. Wachtmeister, C. A. *et al. To be published.*
32. Culberson, C. *Phytochemistry* **5** (1966) 815.

Received February 3, 1967.