(1 g) was then added with vigorous shaking. A precipitate immediately separated. After standing for 24 hours, the yellow precipitate was filtered off, washed and dried. Yield: 2.6 g (calc. 2.8 g). The compound was dissolved in 100 ml boiling chloroform, the solution filtered and cooled in ice. Orange-red (dichromate-coloured) crystals separated. The compound was recrystallised once more from chloroform. It does not melt on heating, but gradually turns black without change of the crystal form. Found: C 17.59; H 1.59; Pt 34.97. Calc. for $[PtI_2(C_8H_8)]$, (553.2) : C 17.37; H 1.46; Pt 35.38.

Molecular weight (cryoscopically in bromoform solution): 492, 610. Dipole moment:

c = 0.00198 molar, $\Delta\varepsilon$ = 0.0130, μ = 7.3D;
c = 0.00406 molar, $\Delta\varepsilon$ = 0.0240, μ = 7.0D.

1. Karle, I. L. *J. Chem. Phys.* **20** (1952) 65.
2. Anderson, J. S. *J. Chem. Soc.* **1936** 1042.
3. Jørgensen, S. M., and Sørensen, S. P. L. *Z. anorg. Ch.* **48** (1906) 441.

Received April 29, 1953.

Release of Hydantoins from Proteins *

HALVOR N. CHRISTENSEN

Carlsberg Laboratories, Copenhagen, Denmark

In a note by the author [1], the procedure of Fraenkel-Conrat and Fraenkel-Conrat [2] for the release of hydantoins from proteins was erroneously quoted, the temperature for the reaction being given at 75° C instead of 36° C (*cf.* Fraenkel-Conrat and Fraenkel-Conrat [3]).

1. Christensen, H. N. *Acta Chem. Scand.* **6** (1952) 1555.
2. Fraenkel-Conrat, H., and Fraenkel-Conrat, J. *Acta Chem. Scand.* **5** (1951) 1409.
3. Fraenkel-Conrat, H., and Fraenkel-Conrat, J. *Federation Proc.* **11** (1952) 214.

* *Correction to* Attempted Successive Applications of the Edman Degradation to Insulin [1].

Received May 4, 1953.

The Occurrence in Lichens of the Folic Acid-, Folinic Acid-, and Vitamin B_{12}-Group of Factors

A. G. M. SJÖSTRÖM and L.-E. ERICSON

Division of Food Chemistry, Royal Institute of Technology, Stockholm, Sweden

In connection with investigations on the folic acid-, folinic acid-, and vitamin B_{12}-group of factors in algae it was considered of interest to study the occurrence of these factors in some lichens, a group of plants known to represent a symbiosis between fungi and algae. The following lichens have been studied using microbiological and bioautographic methods: *Cladonia silvatica, Umbilicaria pustulata, Parmelia physodes, Parmelia furfuracea, Cetraria islandica, Evernia prunastri, Alectoria jubata,* and *Usnea comosa.* The lichens were collected at Grönvik, Nämdö.

Escherichia coli 113-3[1] served as a test organism for the vitamin B_{12}-factors, *Leuconostoc citrovorum* ATCC 8 081[2] for the folinic acid and *Streptococcus faecalis* ATCC 8 043 for the folic acid tests. The organisms were utilized in the agar cup plate method. For *Streptococcus faecalis* the Difco folic acid assay medium with 1.6 % Bacto agar was used [3]. The solvent for the chromatographic separation was sec. butanol saturated with water, and containing 3 % acetic acid and 25 mg KCN/l.

The lichens were carefully cleaned and dried at room temperature. Two grammes of finely ground material were suspended in 25 ml of water or buffer solution. Three different methods of freeing the active substances were tried, namely, extraction with water at 37° C for 24 hrs, autoclaving for 20 min. at 120° C in water containing small amounts of KCN, and enzymatic treatment with a chicken pancreas homogenate at 37° C for 24 hrs at pH 7.5. Autoclaving gave the highest

Table 1. Total activity expressed in μg standard per g dry weight of lichens.

Lichenes *	*E. coli* Vit. B_{12} std.	*L. citrovorum* Leucovorin std	*S. faecalis* Folvite std.
Cladonia silvatica	0.035	0.68	0.26
Umbilicaria pustulata	0.02	0.19	0.24
Parmelia furfuracea	< 0.005	0.09	0.43
Parmelia physodes	0.02	2.35	1.16
Cetraria islandica	< 0.005	1.80	1.16
Evernia prunastri	0.008	0.06	0.26
Alectoria jubata	< 0.005	0.01	0.41
Usnea comosa	0.015	0.28	0.41

* Symbiosis of fungi belonging to *Discomycetes*, order *Lecanorales* and algae belonging to *Chlorophyceae*, order *Chlorococcales*.

values for *E. coli*, enzymatic treatment the highest values for *L. citrovorum* and *S. faecalis*.

The results are given in Table 1.

Table 2 shows the various types of growth factors for *S. faecalis* and *L. citrovorum* as revealed after chromatographic separation of the lichen extracts.

The experiments with *E. coli* showed the presence in the lichens of substances having vitamin B_{12}-like activity in a concentration of from less than 0.005 to 0.035 μg B_{12}/g dry weight. This comparatively low activity was found to be due to three different vitamin B_{12}-factors having the same R_F-values as vitamin B_{12},

Table 2. L. Citrovorum and S. faecalis factors in lichens.

Factors	R_F-values	*L. citrovorum* activity in samples A	ET	*S. faecalis* activity in samples A	ET
Unidentified	0.07			+	+
Pteroic acid	0.10				
Unidentified	0.18		++++	+	+++
Pteroyltriglutamic acid	0.22			+	++
Unidentified	0.24	+	+		
Folic acid	0.27			(+)	(+)
Unidentified	0.37			+	+
Formylpteroylglutamic acid	0.45			+++	
Folinic acid	0.55	++	+	++	+
Formylpteroic acid	0.65			+	++
Thymidine	0.70	+	+	+	+

The number of plus signs indicates the intensity of the growth caused by the different factors as observed after chromatographic separation. A = autoclaved, ET = enzyme treated.

vitamin B_{12s}[4] and factor C[5,6]*, respectively. No evidence was found for the presence of factors having the same R_F-values as pseudovitamin B_{12}[7], vitamin B_{12f}[8], vitamin B_{12m}[9], factor A[5], or factor B[5]. The presence of vitamin B_{12s} is of interest because to date its only other source has been marine algae[4].

For *L. citrovorum* four growth factors were found. Two of these have been identified as the natural citrovorum factor[10,11] (folinic acid) (R_F 0.55) and thymidine (R_F 0.70). The other two factors had R_F-values of 0.18 and 0.24. It seems likely that these factors are identical with two of the citrovorum factors other than folinic acid found in a red marine algae *Furcellaria fastigiata*[12].

Altogether nine substances stimulating the growth of *S. faecalis* were observed. Folinic acid and thymidine, which stimulate the growth of both *S. faecalis* and *L. citrovorum*, and factors with the same R_F-values as pteroyltriglutamic acid (Teropterin, Lederle), N_{10}-formylfolic acid and N_{10}-formylpteroic acid (Rhizopterin) were found. Only traces of folic acid (pteroylglutamic acid) could be detected. The other factors observed had approximately the R_F-values 0.07, 0.18, and 0.37 respectively in this solvent system.

Enzymatic treatment of the extracts did not lead to release of folic acid (pteroylglutamic acid) or to folinic acid (N_5-formyl-tetrahydrofolic acid), but substances with lower R_F-values. It was also observed that the enzyme complex of the chicken pancreas homogenate did not seem to release folic acid from a solution of crystalline pteroyltriglutamic acid (Teropterine, Lederle). This is in agreement with previous findings[14,15]. Instead it converted the pteroyltriglutamic acid to a growth factor for *L. citrovorum* which is not folinic acid. The value of the total activity for *S. faecalis* and *L. citrovorum* in Table 1 do not therefore represent the true folic acid or folinic acid content of the lichens.

Most lichens investigated contained substances having an antibiotic effect on *E. coli*, *S. faecalis* and *L. citrovorum*, possibly due to usnic acid.

The authors wish to express their thanks to Prof. C. A. Elvehjem and to Dr. U. J. Lewis, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin, for samples of vitamin B_{12f} and pseudovitamin B_{12}, to Dr. J. W. G. Porter, The National Institute for Research in Dairying, Shinfield, Reading, for a solution containing factor A and factor B, and to Dr. H. G. Wijmenga, Organon, Oss, for a sample of vitamin B_{12m}.

We are also grateful to Dr. M. Waern, Institute of Plant Ecology, Upsala, Sweden, for botanical classification of the lichens.

1. Diding, N. *Scand. J. Clin. Lab. Invest.* **3** (1951) 215.
2. Bánhidi, Z. G., Sjöström, A. G. M., Grömmer, S., and Lundin, H. *Arkiv Kemi* **5** (1953) 393.
3. Capps, B. F., Hobbs, N. L., and Fox, S. H. *J. Bact.* **55** (1948) 869.
4. Ericson, L.-E. and Lewis, L. *Arkiv Kemi In press.*
5. Ford, J. E., Kon, S. K., and Porter, J.W.G. *Biochem. J. (London)* **56** (1951) ix.
6. Sjöström, A. G. M.. Neujahr, H. Y., and Lundin, H. *Acta Chem. Scand.* **7** (1953) *In press.*
7. Pfiffner, J. J., Dion, H. W., and Calkins, D. G. *Federation Proc.* **11** (1952) 269.
8. Lewis, U. J., Tappan, D. V., and Elvehjem, C. A. *J. Biol. Chem.* **199** (1952) 517.
9. Wijmenga, H. G. *Investigations on vitamin B_{12} and related factors.* Thesis, Utrecht 1951.
10. Sauberlich, H. E., and Baumann, C. A. *J. Biol. Chem.* **176** (1948) 165.
11. Keresztesy, J. C., and Silverman, M. *J. Am. Chem. Soc.* **73** (1951) 5510.
12. Bánhidi, Z. G. and Ericson, L.-E. *Acta Chem. Scand.* **7** (1953) 713.
13. Ericson, L.-E., Widoff, E., and Bánhidi, Z. G. *Acta Chem. Scand.* **7** (1953) *In press.*
14. Dabrowska, W., Kazenko, A., and Laskowski, M. *Science* **110** (1949) 95.
15. Wieland, O. P., Hutchings, B. L., and Williams, J. H. *Arch. Biochem. et Biophys.* **40** (1952) 205.

Received April 9, 1953.

* A factor found in sewage sludge[6] and showing the properties of factor C has been used for comparison.