Studies on the Chemistry of Lichens

VI.* Chemical investigations of the lichen species Alectoria nigricans (ACH.) Nyl. and Parmelia alpicola Th. Fr.

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Parmelia alpicola Th. Fr. and Alectoria nigricans (Ach.) Nyl. have been chemically investigated with special regard to their content of lichen substances. Both the species have been found to contain Alectorialic acid as the main compound and probably Thamnolic acid in trace amounts. In addition to these two acids, mannitol and a tetrahydroxy fatty acid have been isolated from Parmelia alpicola. Further-more three unidentified substances have been detected in the same lichen species by paper chromatographic separation. Alectorialic acid was identified as a depside aldehyde and most likely as a mixed orcinol and β- orcinol derivative. The depside was hydrolysed and the aldehyde-component was found to be Atranol.

During our research on lichen samples, Alectoria nigricans and Parmelia alpicola were investigated. The lichen species Alectoria nigricans was first described in some detail by Zorr, who isolated from it a crystalline lichen substance called Alectorialic acid, m.p. 175 — 176°C. The compound was found to be a depside belonging to the Olivetoric acid type, with the fundamental formula

\[ \text{Formula 1} \]

in which R, R' and R'' represent an H-atom or an aliphatic group. Zorr further described that the compound gave a blood-red colour with bleaching powder, a yellow colour with diluted alkali and with concentrated sulphuric acid, and a purple-red colour with the same reagent.

Asahina reported in an early publication of Alectoria nigricans that the species gave a negative reaction with p-phenylene diamine (PD). In the present work it has been found that the same lichen species collected in Norway give a yellow colour with the same reagent.

In a work by Hess on paper chromatography of lichen substances, Alectorialic acid is mentioned. Further Krog reports of not identified lichen substances from Parmelia alpicola.

As there is no previous report of any systematic chemical study of these Norwegian lichen species, a thorough examination of the lichens has now been made. As a result of these investigations, it has been found that the main compound of these species is Alectorialic acid. The chemical composition of Alectorialic acid is highly interesting and is especially discussed in this paper. A-portion, Molekular-formula 2). Alectorialic acid also gave a deep yellow colour with concentrated sulphuric acid. It has further been stated that the compound does not give the characteristic homofluorescein reaction when heated with chloroform and alkali.

As the Alectorialic acid gives a blood-red coloration with bleaching powder, similar to that described by Zorr, it is to be expected that the depside has free hydroxyls at 2 and 4 positions of the S-portion in the molecule, (see the Molecul-formula 2).

It may be mentioned here that this in the first record made of the occurrence of a depside with all the three colour reactions: Bleaching powder-red, red, yellow.

* Part V: Annales Botanici Fennici 4 [1967], in press.
1 W. Zorr, Die Flechtenstoffe in chemischer, botanischer, pharmakologischer und technischer Beziehung, Jena 1907.
2 Y. Asahina, Jap. J. Bot. 12, 687 [1936].
3 D. Hess, Planta 52, 65 [1958].
4 H. Krog, Nytt Magasin for Naturvidenskaperne 88, 57 [1951].
PD-yellow and alkali-yellow. As ASAHINA reported, depsidones do not colour with bleaching powder.

I have also made use of the colour reaction with 2,6-dichloro-quinone chlorimide test in a 0.1 N sodium borate buffer solution (pH = 9.3), which has been employed by GIBBS to indicate the presence of an H-atom or a COOH-group para to a free hydroxyl in an aromatic ring. In these cases a deep blue colour of the indophenol compound develops instantly. Alectorialic acid gave instantaneously a deep blue colour reaction with the reagent, thus showing a free H-atom para to a free hydroxyl group! In the molecular structure which is illustrated in Formula 2, the conditions at 2 and 5 positions in portion A, or at 2 and 5 positions in portion S if R' is substituted with H or COOH, should give a positive reaction with 2,6-dichloro-quinone chlorimide. To compare, the indophenol-test has been made with the lichen substances Thamnolic acid, Evernic acid and Atranol. All the substances immediately gave the same reaction as did Alectorialic acid.

The action of methanolic potash or trisodium-phosphate solution on Alectorialic acid, surprisingly, did not have any hydrolytic effect, and only the Decarboxy-Alectorialic acid was obtained. This fact supports the theory that the Alectorialic acid ought to be a ß-orcinol-derivate.

In these investigations the lichen Parmelia alponica was collected in the Hovringen region, Sel, Oppland and in Reinheim, Dovre.

The material was only slightly contaminated with the species Alectoria lanata (= Parmelia pubescens), and was collected in July–August 1957. Alectoria nigricans was collected in different parts of the country, and the greatest possible care was taken to avoid contamination by other species, especially by Cornicularia divergens.

Before the extraction the material was purified, dried and ground into coarse powder. The extraction process was carried out first with ether, and subsequently with acetone in a Soxhlet extractor designed to take 400–500 grams of material. Details of the extraction process and the purification of the extracts are described in the experimental part.

Results and discussion

From Alectoria nigricans only Alectorialic acid has been isolated. After recrystallisation of the compound from acetic and ethanol, the acid melted with decomposition, at 175–176°. The melting point is in accordance with the data given by ZOPF. We obtained for the pure Alectorialic acid, C = 57.7% and H = 4.40 percent. C_{22}H_{29}O_{11} requires C = 57.4% and H = 4.38 per cent.

The compound was slightly soluble in ether, chloroform, benzene and cold alcohol (ethanol), more soluble in warm alcohol and in acetic acid. The acid possessed a very bitter taste.

The compound was found to be a carboxylic acid, carbon-dioxide being briskly effervesced from a solution of sodium hydrogen carbonate when the solution was added to it. Treated with a cold mixture of glycerol-ethanol-aniline (2 + 2 + 1), the acid decarboxylates very easily.

p-Phenylene diamine (PD), benzidine and alkali all gave a deep yellow colour with the isolated substance. With 2,4-dinitro-phenylhydrazine an insoluble yellow hydrazone was obtained. The last mentioned reaction and the colouration with PD and benzidine clearly demonstrate the presence of an aldehydic group.

An alcoholic solution of the acid gave a purple-red colour with a drop of ferrec chloride solution, indicating the presence in the molecule of a carbonyl group ortho to a hydroxyl (positions 5 and 6 in the molecular structure illustrated in Formula 2). Complete hydrolysis of the pure depside from Alectoria nigricans was obtained by the action of boiling glacial acetic acid on the Alectorialic acid or by prolonged reflux of the acid with 96% ethanol. On working up the hydrolysis solutions only Atranol was isolated and purified by recrystallisation from water.

The product has been compared with an authentic sample of Atranol which was obtained by hydrolysis of Atranorin by the method of PFAU. See also the infra-red curves in Fig. 2, and the R_f-values in Table 2 where Atranol is marked with the letter A.

I did not succeed in isolating the S-portion of the molecule.

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8 Y. ASAHINA and H. AKAGI, Ber. dtsch. chem. Ges. 68, 1130 [1935].
As mentioned in the introductory text, ZOPF was of the opinion that the Alectorialic acid belonged to the Olivectoric acid group. All substances in this class are orcinol-derivates. On the basis of the results in this work I am not convinced by that idea. Consequently Alectorialic acid was assigned the probable molecular structure containing hydroxyls, one aldehyde group in position A-6 and one carboxyl group in position A-4. No methoxyl group has been detected in Alectorialic acid.

ASAHINA has reported that the carboxyl group in the A-portion of a depside is invariably at para or meta position to the depside linkage. Very likely the 3-position in portion S of the Alectorialic acid is free. 5 and 6-positions in the S-portion are at present unknown.

Alectorialic acid was isolated as the main compound from Parmelia alpicola. Further Mannitol, m.p. 165–167°C, and a Tetrahydroxy fatty acid, m.p. 180–181°C (corr.), were obtained. The yield of the slightly impure Alectorialic acid obtained from this species was about 0.6%, of the pure Mannitol 0.003% and of the pure Tetrahydroxy fatty acid 0.03 per cent. Work is in progress with a view to establishing the constitution definitely of the hydroxy acid. The results of these investigations will be published in the near future in connection with investigations of similar substances isolated from other lichen species.

Ultraviolet and Infra-red Absorption Spectra of the Depside

The identity of Alectorialic acid from Alectoria nigricans and from Parmelia alpicola was confirmed by comparison of the ultraviolet and infra-red absorption spectra, Fig. 1 and Fig. 3. The curves of the acid from the two lichen species were very similar. IR-Spectra were also compared with spectra of Atranorin, and a strange similarity was obtained. Ultraviolet absorption spectra in the readily accessible region above 220 μ of Alectorialic acid from Alectoria nigricans (AN) and from Parmelia alpicola (PA), Thamnolic acid (Tha) from Haematomma ventosum and of Atranorin from Parmelia spec. (Atr.) are presented in Fig. 1. The substances were all dissolved in 96% ethanol in concentration of about 10⁻⁴ M, and the spectra measured to identify and to obtain any information on the chemical
structure. The compounds gave maxima of absorption at the following wavelengths:

- **AN**: 237, 260 and 335 μm,
- **PA**: 238, 260, a slight peak or shoulder at 305, and 330 μm,
- **Tha**: 235, 260, 314 and 350 μm,
- **Atr**: 250 and 317 μm.

Thamnolic acid and Atranorin are both β-orcinol-aldehyde-depsides. These lichen substances demonstrate the good similarity to the Alectorialic acid. In Fig. 1a the absorption curves of Evernic acid, Olivetoric acid, Divaricatic acid and Barbatic acid are shown. The first three substances are all orcinol-derivatives while the Barbatic acid is a β-orcinol-depside.

The absorption maxima for Alectorialic acid occur at wavelengths in good agreement with the results of HALE and GREAM and RIGGS, who have made UV-investigations into lichen depsides and depsidones. For β-orcinol-depsides HALE reported absorption maxima at 238 and 312 μm, for orcinol-depsides at 270 and 307 μm. The β-orcinol-depsides and depsidones give maxima of absorption at 238 and 312 μm with much higher absorbancy in shorter than in longer wavelengths. HALE further reported that increasing numbers of substituents, especially carboxyl or carbonyl radicals, exert a dampening effect on the absorption bands. The author has in mind the weak bands for Alectorialic acid in the region 300 to 350 μm.

As mentioned previously in this paper the Alectorialic acid dissolves in an alkali solution with a yellow colour. The absorption maxima of the yellow solution of Alectorialic acid from the two lichen species and of the solution of Thamnolic acid, are shown in Table 1. Complete similarity was found of the Alectorialic acid from *Alectoria nigricans* and *Parmelia alpicola*. Impurities which were present in the Alectorialic acid from *Parmelia alpicola* did not cause disturbance in UV or in IR absorption region.

<table>
<thead>
<tr>
<th>Compound</th>
<th>In acetate buffer pH = 10</th>
<th>In N NaOH in methanol [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alectorialic acid from A.n.</td>
<td>295–327–415</td>
<td>295–392</td>
</tr>
<tr>
<td>Alectorialic acid from P.a.</td>
<td>295–327–415</td>
<td>295–392</td>
</tr>
<tr>
<td>Thamnolic acid from H.v.</td>
<td>270–323–405</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Alectorialic acid and Thamnolic acid gave maxima of absorption at the following wavelengths. (A.n. = *Alectoria nigricans*; P.a. = *Parmelia alpicola*; H.v. = *Haematomma ventosum*).

Speed of the scan was slow enough to record maxima accurately. Measurements were made within a few minutes after preparation of the solutions. The yellow solutions of the mentioned substances gave the same results measured after 60 minutes.

**Paper Chromatography of the crude Extracts from Alectoria nigricans and Parmelia alpicola**

Crude extracts from the two lichen species were analysed by paper chromatography and mainly according to Hess. n-Butanol-ethanol-water (4 + 1 + 5) and n-butanol-acetone-water (5 + 1 + 2) were used as developing medium in a descending method. Both the solvent systems gave virtually the same results, and the Rf-values are the mean of several analysis.

The air-dried chromatograms were examined in ultraviolet light and the positions of the spots being marked. The papers were then sprayed with bis-diazotised benzidine solution, and with a freshly prepared 0.1% alcoholic solution of PD.

Trace of Thamnolic acid was observed both in *Alectoria nigricans* and in *Parmelia alpicola*. The spots gave a yellow colour with PD and Rf-values were identical with that of Thamnolic acid extracted from *Haematomma ventosum*.

In ultraviolet light three unidentified spots (B, C and D, Table 2) were detected in *Parmelia alpicola*. These three compounds have not given any reaction of sugars, amines, amino acids, carboxylic acids, phenols, aldehydes or sterols. I did not succeed in removing the three compounds by purifying the Alectorialic acid from *Parmelia alpicola*. On hydrolysis of crude extract from *Parmelia alpicola* with glacial acetic acid, the three components B, C and D were found unchanged left in the hydrolysate.

On the paper chromatograms the Alectorialic acid always appeared as two nearby spots (PD+), which in Table 2 are marked with the letters E and F. I am of the opinion that these two spots are identical with the native Alectorialic acid and Decarboxy-Alectorialic acid. Especially on Whatman No. 20, these two compounds separated satisfactorily. Probably the Alectorialic acid decarboxylates during the chromatographic development.

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12 Y. J. SOLBERG, Acta chem. scand. 11, 1477 [1957].
Chromatographic analysis of the extracts on paper impregnated with alkali phosphate buffer or with boric acid, did not give any additional information to that already obtained with analysis on untreated paper.

On chromatographic separation of the extracts from the two lichen species, trace of two components (S and A in Table 2) with high $R_f$-values are always found. One of them (A) was $PD^+$ and identical with Atranol. It is anticipated that the S-spot is identical with the other half of the Alectorialic acid, the S-portion of the molecule in Formula 2. This part of the molecule is still unknown.

It is propable that a certain degree of hydrolysis takes place in the process of extraction or purification of the lichen substances. Further it has been observed that the Alectorialic acid to some extent hydrolyses by standing for a considerable time dissolved in ethanol.

**Experimental procedure**

Melting points were determined on a Kofler micro block. Intra-red absorption spectra were taken in KBr-discs. Ultraviolet absorption spectra were recorded with a Beckman DB recording spectrophotometer. Paper chromatography was carried out on Whatman paper No. 20 or on Ederol paper No. 202. Analytical grade reagents were used for all reactions.

**Extraction of Alectorot nigricans**

The lichen sample (150 g) was extracted by refluxing with ethyl ether in a Soxhlet extractor for about 6 hours. After removing the solvent under reduced pressure from this extract, a residue amounting to 5.9% (8.8 g) of the weight of the lichen was obtained. The residue, a semi-solid light brown mass, was purified by washing repeatedly with cold chloroform and light petroleum (b.p. 40–70°C). A light greenish, crystalline product (7.1 g) was obtained by this procedure. The purification of the crude mixture involved

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Table 2. $R_f$-values at about 22°C of the components in crude extracts from Parmelia alpicola (PA), Alectoria nigricans (AN), of the hydrolysis product from Alectorialic acid and of Atranol. * BEV = n-Butanol-ethanol-water, BAV = n-Butanol-acetone-water. ** Only as traces. Components B, C and D gave strong violet fluorescence in ultra-violet light. Component S gave a weak violet or blue fluorescence in ultra-violet light.

<table>
<thead>
<tr>
<th>Component</th>
<th>PA</th>
<th>AN</th>
<th>Hydrolysis product</th>
<th>Atranol</th>
<th>Colour reaction with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thamnolic acid</td>
<td>0.22</td>
<td>0.22</td>
<td>Yellow</td>
<td>Red</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0.67</td>
<td>0.67</td>
<td>Yellow</td>
<td>Purple-red</td>
<td>Red</td>
</tr>
<tr>
<td>F</td>
<td>0.74</td>
<td>0.74</td>
<td>Yellow</td>
<td>Red</td>
<td>Orange</td>
</tr>
<tr>
<td>S</td>
<td>0.83**</td>
<td>0.81**</td>
<td>Yellow</td>
<td>Red</td>
<td>Violet</td>
</tr>
<tr>
<td>A</td>
<td>0.87**</td>
<td>0.86**</td>
<td>0.93</td>
<td>0.93</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

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some loss of material. Part of the crude product (0.75 g) was then recrystallised from hot acetic acid and finally twice from 60% aqueous ethanol. The colourless needles were filtered and washed with a little light petroleum. The substance obtained melted at 175—176°C, and was identified as Alectorialic acid. Further recrystallisation did not raise the melting point. The purity of the acid was checked by paper chromatography. Final extraction of the lichen material with acetone yielded no crystalline product.

**Extraction of Parmelia alpica**

A total of 515 g of the lichen sample were extracted for 7 hours with ethyl ether in a Soxhlet extractor. On concentrating the extract a light greenish crystalline solid (PA-1) was deposited, filtered off and washed on filter with light petroleum (b.p. 40—70°C) to remove impurities; yield 1.8 g (0.6%). From its properties and reactions the fraction obtained was found to contain the same substance, Alectorialic acid, as isolated from Alectoria nigricans. However, Alectorialic acid from this lichen species was contaminated with a tenacious impurity which rendered the purification extremely difficult. Mixed melting point with a sample of pure Alectorialic acid from Alectoria nigricans was therefore not taken.

After removing the solvent from the ether extract, an amorphous resinous product, amounting to 12.3 g (2.4%) of the weight of the lichen was obtained. This fraction consisted mostly of fatty and waxy matter along with some Alectorialic acid, and was not further investigated.

The lichen residue left after the extraction with ether was extracted with aceton for 20 hours. The aceton extract was evaporated to dryness and the residue treated repeatedly with warm ether, chloroform and ethanol to remove dark coloured impurities. The insoluble product (PA-2) was then macerated with water and filtered. The aqueous filtrate was evaporated to dryness under reduced pressure at 25—30°C. The residue was dissolved in boiling ethanol, discoloured and cleared with activated charcoal, filtered and diluted with the same volume of acetone. On standing in a freezer at −25°C for several days a crystalline colourless product was obtained. Yield 10 mg; m.p. 165 to 167°C. (Found: C = 39.4%, H = 7.6%. Calc. for C_{14}H_{8}O_{6}: C = 39.6%, H = 7.7%). The substance was identified as Mannitol and the mixed melting point with an authentic specimen was undepressed.

The water-insoluble portion of PA-2 was now macerated with normal sodium hydroxide solution, washed on filter with the same solution, water and ethanol, and then dried at about 40°C for 24 hours. The substance was dissolved in boiling acetic acid, treated with activated charcoal and filtered warm. After a short time a white, amorphous product precipitated, (PA-3). It was filtered off, washed on filter with acetic acid, ethanol and ethyl ether and then dried at about 100°C for 4 hours.

The water-insoluble fraction (PA-3) was very small and snow-white. The total amount of the purified compound was about 147 mg and identified as a Tetrahydroxy fatty acid; m.p. 180—181°C (corr.).

**Action of alkaline solutions on Alectorialic acid**

On treatment of Alectorialic acid with methanolic potash (5%) for 2 hours at 40°C in nitrogen atmosphere or with 0.1 M trisodium phosphate solution in nitrogen atmosphere for 8 days at room temperature did not have any hydrolytic effect. On working up the alkaline solution only one component in both cases was obtained, the two substances were recovered in about 80% yield of the starting material. The substances proved identical on paper- (R_f = 0.79) and on thin layer chromatograms (benzene-methanol-acetic acid, 450+80+40) and indicated the presence of a component which was PD+ and which gave a deep red colour with Fast Blue B.

Full proof of their identity was obtained by comparing their infrared spectra in the region 5—10 μ, where both compounds, measured in KBr-discs, were identical in all respects. Complete similarities were also found in the ultraviolet absorption region.

The compound was, in contrast to the Alectorialic acid, easily soluble in cold ether and ethanol, but insoluble in 10% sodium hydrogen carbonate solution. With ferric chloride solution a brown-red or brown-violet colour and with bleaching powder a blood-red colour was obtained. The product was identified as Decarboxy-Alectorialic acid.

**Hydrolysis of Alectorialic acid**

Only the pure Alectorialic acid isolated from Alectoria nigricans was used in the hydrolysis attempts of Alectorialic acid. Reflux of 50—100 mg of the depside with 70 ml glacial acetic acid for 48 hours resulted in complete hydrolysis. After evaporation of the hydrolysis solution in vacuum, the residue was dissolved in 10 ml 96% ethanol and analysed by paper and thin layer chromatography. On the chromatograms only the spot of Atranol was observed, indicated in Table 2 by the letter A.

In the hope of isolating the decomposition products, larger amounts of Alectorialic acid were hydrolysed. On working up the hydrolysis residue, only Atranol was isolated. Recrystallised from water gave a crystalline compound; m.p. 117—118°C. (Found: C = 59.5%, H = 5.51%. Calc. for C_{14}H_{8}O_{6}: C = 59.6%, H = 5.63%). The molecular weight of Atranol (determined by the boiling point elevation method. Calculated for C_{14}H_{8}O_{6} = 152.

Authentic Atranol was prepared from Atranorin by the method of PFAU and purified by recrystallisation from water. The mixed melting point was undepressed.

15 C. A. Wachtmeister, Acta chem. Scand. 6, 818 [1952].
The author wishes to express his indebtedness to Professor Dr. EILIF DAHL, Agricultural College of Norway, Vollebekk, for his interest and advice in this work, and for the collection of the lichen species Parmelia alpicina. I have received a grant from Nansen- fondet - Oslo, which is gratefully acknowledged. The elementary analyses have been carried out by Mikroanalytlaboratoriet, Medicinsk Kemiska Institutionen, Uppsala Universitet, and the IR spectra by Sentralinstitut for industriell forskning, Oslo.

NOTIZEN

Druckinduzierte Gitterstörungen in Kaolinit

KLAUS-JÜRGEN RANGE und ARMIN WEISS
Institut für Anorganische Chemie der Universität München


Unsere Versuche wurden in einer Hochdruckapparatur vom „simple-squeezer“-Typ 3 durchgeführt. Wir fanden, daß durch Drucke von 1 — 15 kbar, die vorwiegend in Richtung der Plättchenormalen einwirken, in wenig fehlgeordneten, morphologisch gut ausgebildeten Kaolinen (Beispiel: Kaolin Georgia well 5) starke Gitterstörungen induziert werden, ohne daß eine wesentliche Änderung der Kristallmorphologie eintritt. Die Gitterstörungen bestehen aus statistischen Verschiebungen der Kaolinitschichten in Richtung der b-Achse mit einem Verschiebungsbetrag von \( n \cdot b / 3 \) (\( n \) = ganze Zahl) und sind beim Tempern (100 °C, 30 Tage) teilweise reversibel (Abb. 1). Eine Veränderung in Lage oder Schärfe der 001-Interferenzen ist nicht nachzuweisen.


In Kaolinen mit starken natürlichen b-Achsen-Fehlordnungen (Beispiel: Kaolin Georgia poor 6) werden

\[
\begin{align*}
Q_S & = 0.75 \\
& = 0.70 \\
& = 0.65 \\
& = 0.60 \\
& = 0.55 \\
& = 0.50 \\
& = 0.45 \\
\end{align*}
\]

Abb. 1. Abhängigkeit des röntgenographischen Fehlordnungsgrades gepreßter Proben des Kaolins Georgia well vom angewandten Preßdruck unmittelbar nach der Druckbehandlung (o — o) sowie nach 30-tägiger Temperung bei 100 °C (□ — □ — □). Der Fehlordnungsgrad ist hier durch die empirische Größe \( Q_S \) gekennzeichnet. Diese setzt die Intensität der Interferenz (111) in Beziehung zum Intensitätsabfall zwischen den Interferenzen (111) und (002). Sie wird an anderer Stelle 6 ausführlich diskutiert.

4 Der Deutschen Forschungsgemeinschaft sei auch an dieser Stelle für die Gewährung einer Sachbeihilfe gedankt.
5 Georgia Kaoline Company, Elizabeth, N.J., USA.