of the C-terminal amino acid (alanine) in this experiment was 82 % compared to 62 – 77 % when the hydrogen fluoride procedure described above was used. Experiment 6 was performed with one equivalent each of Boc-glycine, phenylalanine, asparagine and glutamine *p*-nitrophenyl ester in dimethylformamide for 24 h. The low total incorporation value in this case is supported by a determination [6] of the quantity of free amino groups on a resin aliquot. The value thus obtained was 10 %.

Because of the limited number of experiments performed so far we would like to restrict our comments of the results to the following points. Of the Boc-amino acids used in this investigation, isoleucine and valine have a far lower reactivity than the others. This seems to be in agreement with the experiences of Li and Yamashiro [2] and to some extent of Yajima, Kawatani and Watanabe.[3] On the other hand Boc-leucine does not seem to present special difficulties as reported by the last mentioned authors. With the low reactivity of Boc-isoleucine and -valine in mind we performed the experiments with valine-resin of Table 2, *i.e.* used valine as an amino component in the coupling step. Total as well as relative incorporation values seem to be very similar when alanine- and valine-resins are used, though of course we are aware of the fact that in the first context the experimental error is much too large to permit reliable conclusions. Further work following this approach will show if the nature of the amino component has any influence on the coupling step. Since after all it is a well-known fact that isoleucine and valine are sterically hindered,[7] our experiments demonstrate above all how similar the reactivity is for the other amino acids studied, with the exception of *O*-benzyl-threonine which, like isoleucine and valine, has two β-substituents.

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Chlorinated Long-chain Fatty Acids

Their Properties and Reactions. IV.[1] Quantitative Gas Chromatography of Chlorinated Octadecanoic Acids Prepared from Oleic, Linoleic and Linolenic Acids

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Resin constituents, *i.e.* compounds extracted by neutral organic solvents, that remain in pulp are responsible for serious disturbances during the pulping process and they often have a decisive effect on the quality of the pulp and determine whether it is suitable for conversion into paper or for use as a raw material.[2] For this reason, the deresination of pulp is a very important process, although it is often difficult, especially when the pulp is made from hardwoods like birch.

The main constituents of the fatty acid fractions of the resins of birch, pine, and

spruce woods are unsaturated fatty acids. In stored pulp wood, they occur partly as free acids and partly as esters of glycerol and higher steroid or terpenoid alcohols.[3] The bleaching process, *i.e.* the treatment of pulp with chlorinating and oxidizing agents, is mostly designed to remove the residues of lignin and hemicellulose from the pulp. Unfortunately, the process has an incidental effect on the resin constituents. Since pulp contains appreciable amounts of resin constituents that cannot be removed easily, it is important to know how the resin components are distributed between the bleaching liquors and the pulp. The effects of various bleaching agents on model compounds have been widely investigated, but very little attention has been paid to the development of analytical methods for the determination of the chlorinated and oxidized constituents formed during the pulp bleaching stage, by which the deresination could be followed.

We now wish to report a gas chromatographic method for the quantitative determination of some chlorinated long chain fatty acids (octadecanoic acids) developed in connection with a study of the deresination during the sulfate pulping of birch (*Betula verrucosa* and *Betula pubescens*).

A Varian Aerograph 200 gas chromatograph equipped with a flame ionization detector was used. The peaks were registered with a Philips Recorder Model PM 8000. The gas chromatograph was fitted with a coiled 6.5 ft × 1/8 inch glass column, containing 1 % SE-30 on acid-washed Chromosorb W (80/100 mesh) treated with dimethylchlorosilane. The column was preconditioned by heating it for 24 h at a temperature 10°C higher than the maximum working temperature (300°C) while helium gas was passed through it at a flow rate of about 10 ml/min. Helium was used as carried gas at a flow rate of 40 ml/min in the analytical work. The separations were effected by raising the column temperature from 230° to 290°C at a rate of 5°C/min. The temperature of the injector was held at 290°C and that of the detector unit at 300°C. The electrometer sensitivity was set at range 1 and the attenuation at 32, 64, or 128 depending on the injected amount of substances. The methyl esters of three chlorinated fatty acids, namely, 9,10-dichloro-, 9,10,12,13-tetrachloro-, and 9,10,12,13,15,16-hexachlorooctadecanoic acids were studied. The acids had been prepared earlier by Pihlaja and Ketola by chlorinating oleic, linoleic, and linolenic acids.[1a] Methyl octadecanoate

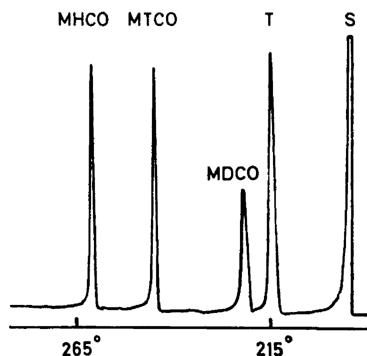

Fig. 1. Gas chromatogram of a mixture of 10.0 mg of tetracosane (T), 9.5 mg of methyl 9,10-dichlorooctadecanoate (MDCO), 10.9 mg of methyl 9,10,12,13-tetrachlorooctadecanoate (MTCO), and 10.6 mg of methyl 9,10,12,13,15,-16-hexachlorooctadecanoate (MHCO) dissolved in 10 ml of tetrahydrofuran (S).

(designated MO) and tetracosane were obtained from Applied Science Laboratories, Inc., USA.

The responses of the flame ionization detector to the chlorinated alkanoates were studied by injecting samples containing different amounts of the methyl esters in nearly the same ratios into the gas chromatograph. The samples were prepared by weighing appropriate amounts (12 – 24 mg) of the esters into vials, which could be closed airtight. Tetracosane was added as an internal standard to every sample. The weighed material was dissolved in a measured volume (5 – 25 ml) of redistilled tetrahydrofuran so that a desired concentration (0.5 – 5 mg/ml) was achieved. The analyses were carried out by injecting 1 μl samples of each solution into the column 3 – 6 times in succession. The injections were made immediately after the preparation of the samples.

The retention values were measured from the front of the solvent peak to the maxima of the peaks of the substances in question. The peak areas were measured by triangulation or by a Disc Integrator coupled to a Disc Model 610 Automatic Printer.

Several column packings were tested for their ability to separate the chlorinated fatty acids.[1a] The SE-30 column was selected for the analyses because of its good separation power, availability and thermal stability. Similar results can also be obtained with a 1 % XE-60

column. The retention times of the compounds in question relative to the retention value of tetracosane are given in Table 1. Possible

Table 1. Relative molar response factors (R) for the quantitative determination of chlorinated octadecanoic acids by gas chromatography as their methyl esters on an SE-30 column.

Compound	Relative retention time	R	Molecular weight
Tetracosane	1.00	1.00	338.7
MO[b]	0.40	0.97 ± 0.04[a]	298.5
MDCO[c]	1.18	0.56 ± 0.03	367.4
MTCO[d]	1.96	0.50 ± 0.02	436.3
MHCO[e]	2.86	0.48 ± 0.05	505.2

[a] Standard deviation based on at least 9 determinations.
[b] Methyl octadecanoate.
[c] Methyl 9,10-dichlorooctadecanoate.
[d] Methyl 9,10,12,13-tetrachlorooctadecanoate.
[e] Methyl 9,10,12,13,15,16-hexachlorooctadecanoate.

sample decomposition or strong adsorption on the support was checked by injecting different dilutions of a sample containing both tetracosane and the methyl esters of the chlorinated fatty acids. The ratios of the different peak areas remained constant and hence indicated that no appreciable decomposition or support interaction had occurred.

Table 1 presents relative molar response factors for the studied compounds calculated from the equation [4,5]

$$R = A_e W_s / W_e A_s \qquad (1)$$

where A_s and A_e denote the peak areas of the standard and the esters in question, and W_s and W_e denote the corresponding amounts (mg) of injected standard and ester. The relative molar response factors show that the response of the flame ionization detector decreases with increasing number of chlorine substituents. The decrease is most marked between methyl octadecanoate and methyl 9,10-dichlorooctadecanoate, whereas no appreciable differences are observed between the correction factors for methyl octadecanoate and its unsaturated, non-chlorinated homologs reported by Nestler and Zinkel.[5] Ongkiehong [6] has pointed out that the response of the hydrogen flame ionization detector is proportional to the weight percentage of carbon in organic substances but that the sensitivity decreases as soon as atoms other than carbon and hydrogen are introduced into the molecule. This decrease cannot be regarded as constant for each hetero atom. For this reason he stated that the detector should be calibrated for every component. This statement is in agreement with the findings in this work. Obviously, the chlorine content of the molecule greatly affects the response of the flame ionization detector and tends to lower it. Consequently, a high accuracy can be achieved in gas chromatography of mixtures of esters of different chlorinated fatty acids by a proper identification of each component in the sample and quantification based on the relative molar response factors. In practice, it is, however, possible (Table 1) to use approximate R values, 1.0 for methyl octadecanoate and 0.5 for its chlorinated derivatives, when the formation of chlorinated long-chain fatty acids during the bleaching of pulp is being studied.

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