SUMMARY

1. The process of the splitting out of N-acetylglucosamine in the hydrolysis of myelomic IgM and its $(Fc)_5$ and Fab fragments with 3 N HCl at 100, 110, and 120°C has been investigated as a function of the time.

2. The maximum splitting out of N-acetylglucosamine from IgM and the $(Fc)_5$ and Fab fragments takes place at 110°C in 4, 6, and 8 h, respectively.

3. The amounts of N-acetylglucosamine found in the preparations studied agree well with the primary structure of the oligosaccharide groups of IgM.

LITERATURE CITED

1. J. Montreuil and G. Spik, Microdosage des Glucides, Monographie, Lille 2 (1968), p. 195.
2. V. A. Lapuk, F. V. Shmakova, and E. D. Kaverzneva, Bioorg. Khim., 1, 1134 (1975).
3. J. Baenziger and S. Kornfeld, J. Biol. Chem., 249, 7270 (1974).

# THE STRUCTURE OF KENAF LIGNIN

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Previously [1, 2], by successive acidolysis three fractions of dioxane lignin (DLAK-I, DLAK-II, and DLAK-III) were isolated from the stems of kenaf of the variety "Uzbekskii 15-74," their chemical compositions were determined, their semiempirical formulae were deduced, and their molecular-weight distributions and the products of their nitrobenzene oxidation were studied.

In the present work we considered the structure of fractions of kenaf DLA isolated by the method of reductive degradation with metallic sodium in liquid ammonia.

As is well known [3-8], under the action of alkali metals in liquid ammonia on natural lignin and also on lignin isolated from wood, the alkyl-aryl ether bonds between the phenylpropane structural units (PPSEs) present in them are cleaved. This leads to the liberation of "uncondensed" structural elements.

The fractions were cleaved by the method described in the literature [3-6], and the reaction products were separated by extraction with ether from alkaline solutions (pH 8) and with ether and chloroform from acid solutions (pH 2). The yields of phenols in a single treatment of the DLAKs were 21.5, 20.7, and 19.8% (on the lignin taken), respectively. The yields of phenols from spruce LMR* [8] was 19%. As can be seen, the maximum yield of ether-soluble phenols was obtained from DLAK-I, and it fell successively on passing to DLAK-III. This agrees well with the semiempirical formulae of these lignins [1], where in DLAK-I there are 0.75 alkyl-aryl bonds per $C_9$ and DLAK-III only 0.20. Consequently, there are somewhat more "uncondensed" aromatic units in DLAK-I than in two other fractions of kenaf lignin.

The higher yield of low-molecular-weight cleavage products from kenaf lignin as compared with spruce LMR indicates a lower degree of condensation of the kenaf lignins. This is apparently connected with the fact that in the latter there are fairly large amounts of syringyl structural elements [2] in which the fifth position of the aromatic ring is occupied by a methoxy group and, consequently, does not take part in the condensation processes proceeding both in the formation of lignin and as the result of various chemical actions during its isolation from the plant tissue.

To investigate the complex mixture of phenols isolated after cleavage with metallic sodium in liquid ammonia we used gas–liquid chromatography. In the alkaline fraction (pH 8) among the cleavage products we succeeded in identifying phenols of the p-coumaryl, guai-

*The exact expansion of this acronym could not be ascertained. It probably means "lignin from mechanical disintegration" or the like.

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acyl, and syringyl types. The amounts of phenolic substances in the decomposition products of the fractions of kenaf DLA were as follows (% on the mixture):

| Substance | DLAK-I | DLAK-II | DLAK-III |
|---|---|---|---|
| Phenol (I) | 2.64 | — | — |
| p-Hydroxyphenylpropane (II) | 2.19 | 3.48 | 4.99 |
| 1-(p-Hydroxyphenyl)propan-1-ol (III) | 2.38 | — | — |
| 3-(p-Hydroxyphenyl)propan-1-ol (IV) | 3.53 | 9.74 | — |
| Guaiacol (V) | 1.20 | 0.36 | — |
| Guaiacylpropane-1,3-diol (VI) | 0.75 | 0.41 | — |
| Vanillyl alcohol (VII) | 0.49 | 0.41 | — |
| Guaiacylethane (VIII) | 1.25 | 2.12 | 2.44 |
| 1-Guaiacylethanol (IX) | 0.47 | — | — |
| Guaiacylpropane (X) | 13.12 | 14.81 | 19.86 |
| Vanillin (XI) | 19.54 | 27.00 | 19.08 |
| 1-Guaiacylpropan-1-ol (XII) | 14.34 | 0.74 | 18.64 |
| 3-Guaiacylpropan-1-ol (XIII) | — | — | 4.61 |
| Syringylpropane (XIV) | 22.11 | 37.86 | 18.62 |
| 1-Syringylpropan-ol (XV) | 2.54 | 2.65 | 4.72 |
| Unidentified | 13.38 | 0.43 | 7.05 |
| p-Coumaryl:guaiacyl:syringyl ratio | 0.2:1:0.5 | 0.3:1:0.9 | 0.08:1:0.4 |

A comparison of the compositions of the cleavage products of the three DLAK fractions showed qualitative and quantitative differences between them. In DLAK-I fourteen substances were found, in DLAK-II eleven, and in DLAK-III only eight. These differences can apparently be explained, on the one hand, by the heterogeneity of the distribution of lignin in the plant tissue [9] and, on the other hand, by the localization of these fractions in morphological elements of the tissue with different degrees of accessibility for the reagent. However, the influence of more prolonged acidolysis is not excluded, either.

As can be seen from the figures given above, the ratio of guaiacyl and syringyl structures changes on passing from DLAK-I to DLAK-III: in DLAK-I there is one syringyl structure to two guaiacylpropane structures, and in DLAK-III there is one syringyl to three guaiacyl. In DLAK-II this ratio is 1:1, which is rather difficult to explain.

The comparatively high yields of guaiacyl- and syringylpropanes show that during the reaction the elimination of hydroxy groups in the $\alpha$ and $\gamma$ positions of the side chains takes place. This has been confirmed previously by experiments with model compounds [10]. The detection of phenols (III and IV, and XII and XIII) in the reaction products shows the presence of the initial lignin of p-hydroxyphenyl- and guaiacylpropane units with free OH groups in the side chains in the $\alpha$ and $\gamma$ positions to the aromatic nucleus. Similarly, the detection of phenol (XV) shows the presence of syringylpropane units with free OH groups in the $\alpha$ position to the nucleus in the macromolecules of the initial lignins.

Among the cleavage products, in addition to compounds with a propane chain, phenols with one and two carbon atoms in the side chains were detected, these apparently being products of secondary transformations in reductive degradation. The explanation of their formation may be similar to that given by Yamaguchi [10]. It may be noted that the amount of ethane structures rises from I to III, their amount in DLAK-III being 1.5 times greater than DLAK-I. This is probably the consequence of the more prolonged acidolysis.

## EXPERIMENTAL

Reductive Degradation of Fractions of Kenaf DLA with a Solution of Metallic Sodium in Liquid Ammonia. A sample of DLAK (0.3-1 g) dried in a desiccator over $P_2O_5$ was placed in a reaction vessel, and liquid ammonia (100-150 ml) was added. Metallic sodium cut into small pieces (75% on the weight of the lignin) was gradually added to the stirred mixture. It was then left in a Dewar vessel at −33°C under atmospheric pressure with periodic stirring. After the complete decoloration of the blue solution (reaction time 24 h), the ammonia was evaporated off to dryness and the reaction mixture was freed from the last traces of ammonia by the passage of a current of dry nitrogen. The pulverulent mass remaining in the vessel was

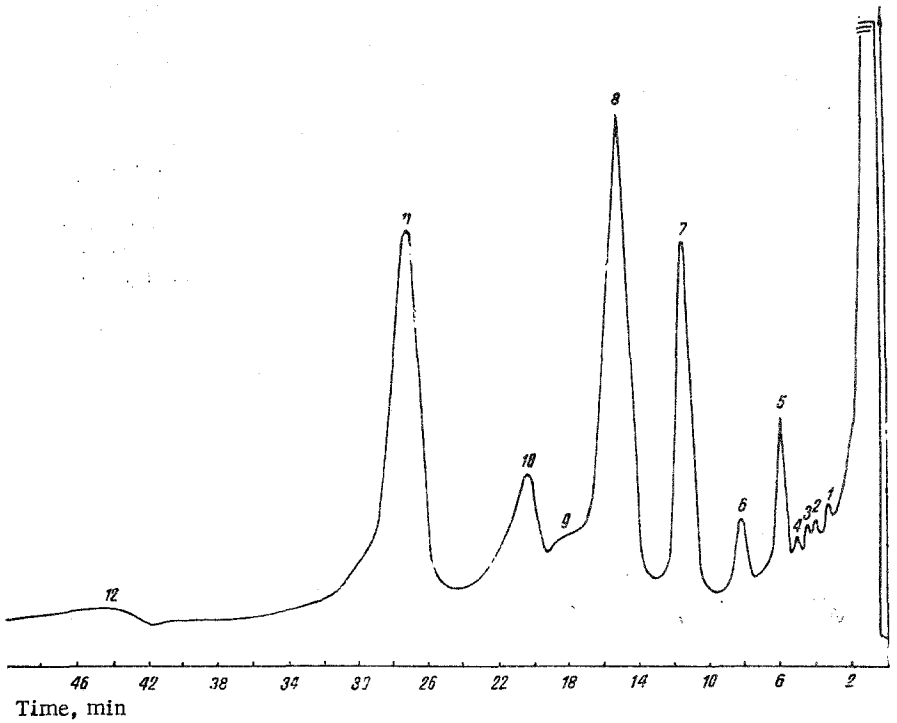


Fig. 1. Gas–liquid chromatogram of the cleavage products of DLAK-II: 1) guaiacol; 2) guaiacylpropane-1,3-diol; 3, 4) vanillyl alcohol; 5) p-hydroxyphenylpropane; 6) guaiacyl ethane; 7) guaiacylpropane; 8) vanillin; 9) 1-guaiacylpropan-1-ol; 10) 3-(p-hydroxyphenyl)propan-1-ol; 11) syringylpropane.

treated with moist ether and then with water (200 ml). The alkaline solution was neutralized with gaseous $CO_2$ to pH 8. The aqueous solution with the wash-waters was exhaustively extracted with ether, dried over calcined $MgSO_4$, and concentrated to dryness. The residue was dissolved in ethanol and was investigated by the GLC method.

Conditions of GLC. Chromatographic analysis was performed on a "Khrom-4" instrument with a flame-ionization detector and a stainless-steel column (370 × 0.3 cm) filled with 15% of Apiezon L on Chromaton NAW DMCS (0.16-0.20 mm). The temperature of the column was 205°C and that of the evaporator 255°C, and the rate of flow of carrier gas (helium) was 40 ml/min. The cleavage products were identified from their retention times and by the addition of markers. Quantitative estimation was carried out by the area standardization method [11].

## SUMMARY

1. Fractions of kenaf DLA have been cleaved by metallic sodium in liquid ammonia. By GLC among the cleavage products have been found phenols the structure of which indicates the presence in the initial lignins of p-hydroxyphenyl-, guaiacyl-, and syringylpropane units with free OH groups in the α and γ positions of the side chain of the aromatic nucleus.

2. It has been found that with the passage from DLAK-I to DLAK-III the number of phenolic substances obtained on cleavage decreases, and the amount of syringyl structures decreases simultaneously, which may be due both to the heterogeneity of the distribution of the lignin in the plant tissue and to its localization in morphological elements of the latter accessible to the reagent to different degrees.

## LITERATURE CITED

1. N. Ya. Kul'chik, G. N. Dalimova, and Kh. A. Abduazimov, Khim. Prirodn. Soedin., 637 (1978).
2. A. A. Geronikaki, G. N. Dalimova, and Kh. A. Abduazimov, Khim. Prirodn. Soedin., 641 (1978).
3. N. N. Shorygina, T. Ya. Kefeli, and A. F. Semechkina, Dokl. Akad. Nauk SSSR, 5, 689 (1949); Zh. Org. Khim., 29, 1558 (1949).
4. N. N. Shorygina and A. F. Semechkina, Zh. Obshch. Khim., 28, 3265 (1958); Trudy ILP i KhD Akad. Nauk LatvSSR, 19 (1960).

5. A. F. Semechkina and N. N. Shorygina, in: The Chemistry of Wood [in Russian], Vol. 1, Riga (1968), p. 57; Izv. Akad. Nauk SSSR, Ser. Khim., No. 5, 887 (1964).
6. Kh. R. Niyazov and N. N. Shorygina, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, No. 3, 563 (1963).
7. N. N. Shorygina and T. Ya. Kefeli, Zh. Obshch. Khim., 20, 1199 (1950).
8. A. F. Semechkina and N. N. Shorygina, Zh. Obshch. Khim., 23, 593, 1593 (1953).
9. B. J. Fergus and D. A. J. Goring, Holzforschung, 24, No. 4, 113 (1970).
10. A. Yamaguchi, Mokuzai Gakkaishi, 19, No. 3, 141 (1973).
11. H. McNair and E. Bonelli, Basic Gas Chromatography, Varian Aerograph, Walnut Creek, Calif. 4th ed. (1968).

# METHODS OF ANALYZING PROSTAGLANDINS

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In recent years, the number of publications devoted to the prostaglandins (PGs) and of patents and investor's certificates connected with this comparatively recently discovered class of natural physiologically active compounds have been constantly rising. A large number of reviews have been devoted to the prostaglandins, but only in some of them [1-6] are methodical approaches to the analysis of PGs considered, and there are no special reviews including all the analytical methods for their investigation.

The aim of the present paper is to cover in condensed form all methods of analyzing PGs.

## Method of Analyzing the Physiological Activity of the Prostaglandins

For a long time, the only method of determining the physiological activity of the PGs and their amounts in tissues under investigation was the use of their capacity for acting on the smooth musclature (stomach, intestine, large intestine, uterus), the blood pressure, etc. [1]. All methods of determining PGs using isolated sections of the smooth musculature have a low specificity for the individual types of PGs, but are fairly sensitive and permit them to be detected in concentrations of $1 \cdot 10^{-8}$ to $50 \cdot 10^{-8}$ g/ml (rat uterus) and less than $10^{-9}$ g/ml. More recently, a series of biochemical methods has been developed of which the following must be mentioned:

a) The enzymatic method, consisting in the oxidation of the 15(S) alcoholic hydroxyl of a PG with purified prostaglandin-15(S) dehydrogenase (from porcine lung) in the presence of $NAD^+$ and the spectrometric detection of the NADH formed [7, 8] (sensitivity about $3.5 \cdot 10^{-10}$ g). The method is not specific for individual types of PGs and is inapplicable to PGB;

b) a method of determining the change in the amount of c-AMP in the cells of the mouse ovary under the action of PGs and their analogs [9], based on determining the amount of labeled c-AMP [10, 11] formed on the incubation of the ovary tissue with [$8^{14}$-C]adenine;

c) a method of determining the affinity (capacity for binding) of the compound under investigation with prostaglandin receptors — rat lipocytes; the results are expressed in the number of nanograms of test compounds displacing 1 ng of labeled PGE per binding section [12].

A radioimmunological method is also distinguished by very high sensitivity ($10^{-10}$ g) [13-17], but the antibodies to the prostaglandins obtained in this way do not possess selectivity, which interferes with the identification of the individual types of PGs.

## Chromatographic Methods of Separating and Identifying the Prostaglandins

Methods of separating the PGs have been described in special reviews [3, 5] and they are considered in fairly great detail in other reviews [2, 4]. In working with the PGs,

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