SUMMARY

L-Asparaginase immobilized on soluble CM-cellulose has been obtained, and some of its physicochemical properties have been studied.

It has been established that L-asparaginase bound to soluble CM-cellulose possesses a greater heat stability and a greater resistance to proteolytic enzymes than the native L-asparaginase.

LITERATURE CITED

1. A. S. Karsakevich, R. A. Zhagat, V. Yu. Son, Khim. Prir. Soedin., 639 (1976).
2. F. Michael and I. Ewers, Z. Macromol. Chem., 3, 200 (1949).
3. O. B. Kinstler and L. V. Kozlov, Biokhimiya, 42, No. 8, 1674 (1977).
4. V. P. Torchilin, I. L. Reizer, E. G. Tishchenko, E. V. Il'ina, V. N. Smirnov, and A. I. Chazov, Bioorgan. Khim., 2, No. 12, 1687 (1976).
5. J. Kirschbaum, J. C. Wriston, and O. T. Ratych, Biochim. Biophys. Acta, 194, 161 (1969).
6. M. R. Pormale, N. A. Kashkina, and É. I. Veinberg, Izv. Akad. Nauk Latv.SSR, Ser. Khim., No. 4, 428 (1972).
7. R. A. Zhagat, I. K. Shprunka, I. A. Éiduse, I. A. Vina, and D. Ya. Daiya, Izv. Akad. Nauk Latv.SSR, Ser. Khim., 73 (1972).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
9. B. J. Davis, Ann. N. Y. Acad. Sci., 121, 404 (1964).
10. R. A. Zhagat, M. R. Buka, and D. A. Kuunyi, Vopr. Med. Khim., 18, 656 (1972).

A STUDY OF THE STRUCTURE OF THE DIOXANE LIGNINS OF THE COTTON PLANT OF VARIETY 108-F BY PROTON MAGNETIC RESONANCE

N. A. Veksler, K. L. Seitanidu,
L. S. Smirnova, Kh. A. Abduazimov,
and M. R. Yagudaev

UDC 547.621:032.11

Analysis of the PMR spectra of acetylated dioxane lignins of the cotton plant collected in various vegetation periods have shown that the DLAs have different degrees of substitution of the C_3 side chain and different degrees of condensation through the aromatic nuclei. With an increase in the time of extraction of the dioxane lignins from the plant, condensation of the lignins isolated takes place. It has been shown that in the DLAs studied the amounts of α-alcoholic hydroxyls are different.

At the present time, in spite of considerable experimental and methodological difficulties [1], the method of high-resolution proton magnetic resonance (PMR) is being used successfully in the study of various lignin preparations.

We have studied the PMR spectra of the dioxane lignins of the stems of the cotton plant of variety 108-F collected in the early period of vegetation (DLA-I) and in the flowering stage (DLA-II), of the ripe stems of the later vegetation period (F-I), and of ripe bolls (DLA-K), and also the dioxane lignins of ripe stems of the cotton plant obtained with different times of extraction (F-II, 2 h; F-III, 3 h). The isolation and characterization of the dioxane lignins has been described in previous papers [2-4]. We have recorded the spectra of the acetylated lignins since they give more valuable information.

In order to assign the chemical shifts of the protons in the PMR spectra of the dioxane lignins a correlation has been made of them with literature information [5-7]. For this pur-

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 388-392, May-June, 1979. Original article submitted December 19, 1978.

0009-3130/79/1503-0337$07.50

TABLE 1. Distribution of the Protons in the Phenylpropane Units of Cotton-Plant DLA*

Zone	Boundaries of the zone, ppm	DLA-I		DLA-II		DLA-K		F-I		F-II		F-III	
		A	B	A	B	A	B	A	B	A	B	A	B
I	2,0—3,7	11,47	1,75	13,38	2,06	13,78	2,03	17,27	2,34	14,98	2,02	15,35	1,95
II	3,7—4,2	2,40	0,38	2,94	0,45	3,57	0,52	4,24	0,58	3,35	0,45	3,49	0,44
III	4,2—4,8	3,62	0,55	2,47	0,38	1,53	0,22	2,36	0,32	2,44	0,33	2,29	0,29
IV	4,8—7,5	30,29	4,63	35,10	5,40	26,02	3,84	45,05	6,12	53,04	7,13	42,59	5,41
IVa	OCH_3 protons†	9,22	1,41‡	14,23	2,19‡	15,05	2,22‡	27,16	3,69‡	27,40	3,69‡	26,93	3,42‡
	Remaining protons	21,07	3,22	20,87	3,21	10,97	1,62	17,89	2,43	25,64	3,44	15,66	1,99
V	7,5—7,9	20,20	3,09	11,76	1,81	18,88	2,78	7,06	0,95	7,21	0,97	7,80	0,99
VI	7,9—8,4	16,96	2,59	25,14	3,86	30,10	4,44	21,35	2,90	17,18	2,31	23,38	2,97
VII	8,4—9,5	14,92	2,28	9,20	1,42	6,12	0,90	2,67	0,36	2,13	0,29	5,02	0,64
	Total	100	15,29	100	15,39	100	14,75	100	13,58	100	13,46	100	12,70

*A is the percentage proportion of the signal and B is the number of protons per C_9 unit.
†The OCH_3 region ranged between the limits of 6.0-6.8 ppm (region IVa in the spectrum).
‡The values found from empirical formulas are given.

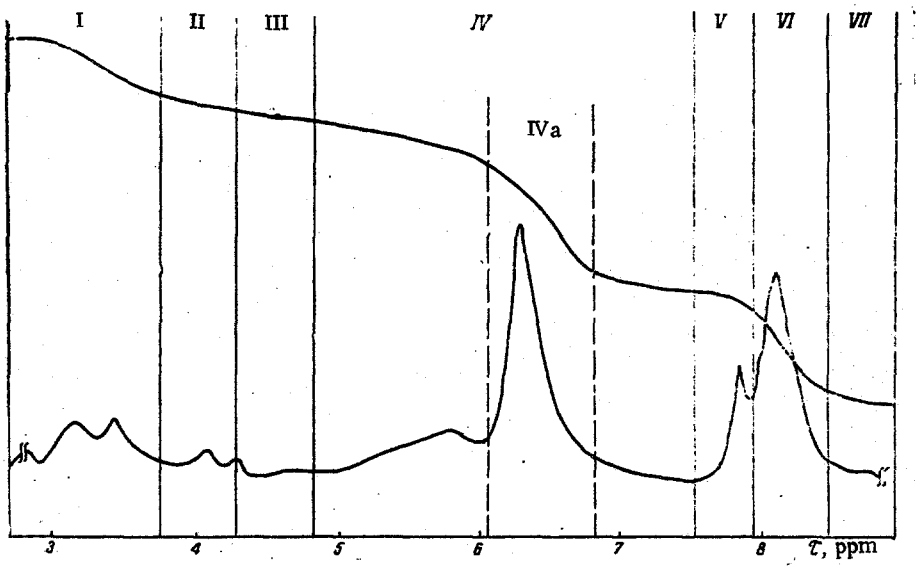

Fig. 1. PMR spectrum of the dioxane lignin of ripe stems of the cotton plant (F-1).

pose, the spectra of the lignins investigated were divided into seven regions corresponding to definite types of protons.

For the quantitative evaluation of the PMR spectra of lignins, the total number of protons in the lignin per elementary C_9 unit is generally calculated from analytical results [5, 8], or a method based on the introduction of an internal standard is used [8]. Our principle is as follows. The total integral intensity of the signals of all the protons was taken as 100%, and then the percentage of the intensity due to methoxyl protons (appearing most clearly in the spectrum of zone IVa) was determined. The percentages of the intensity falling in each integral curve (Fig. 1) and corresponding to definite types of protons were determined similarly. Knowing, from empirical formulas, the numbers of methoxy groups per C_9 unit, the area of the signal of the OCH_3 groups in percentages (zone IVa) per proton was calculated. From these results, the number of protons in each region was established (Table 1).

Since in the PMR spectra of the lignins investigated there were no signals appearing below 2.0 ppm, the boundary of the first zone was taken between the limits 2.0-3.7 ppm. According to the literature in natural lignins there is an insignificant amount of conjugated α-vinyl groups (about 0.06 per C_9 unit) [5]. Thus, it is mainly the aromatic protons that appear in zone I. As can be seen from Table 1, in the cotton-plant DLA from various periods of vegetation the numbers of aromatic protons and, consequently, the degrees of substitution of the aromatic nuclei are different. From the number of free aromatic protons they can be arranged in the following sequence: F-I>DLA-II>DLA-K>DLA-I. Assuming that the number of methoxy groups in them are subject to the same laws, it may be concluded that F-I is the least condensed sample and DLA-I the most.

From a comparison of the numbers of aromatic protons (Table 1) for F-I, F-II, and F-III, it follows that with an increase in the time of extraction the degree of condensation of the lignins isolated rises. The most condensed is F-III, since it contains the smallest number of aromatic protons and OCH_3 groups per C_9 unit.

To calculate the number of condensed units from the PMR spectra of the lignin preparations we used the method suggested by Simionescu et al. [7]. In our calculations we started from the fact that in the DLA of ripe cotton-plant stems (F-I) the amount of p-coumaroyl structural units can be neglected, since their amount is 10-15 times smaller than that of the guaiacyl and syringyl units [9]. On the basis of the OCH_3/C_9 ratio we found for F-I, F-II, and F-III the ratios of guaiacyl and syringyl structures per C_9 unit and, from this, the number of aromatic protons in the nuclei if these nuclei were condensed, i.e., the theoretical number of aromatic protons. (See the display at top of next page.)

From the difference between the number of aromatic protons found theoretically and from the PMR spectra we calculated the degree of structural condensation of the lignins. For example, the total number of condensed units in F-I $(2.77 - 2.34) \cdot 100\% = 43\%$. Analysis of the

Preparation	OCH_3/C_9	Number of aromatic protons		Percentage of condensed nuclei
		Theoretical	from PMR	
Ф -I	1,23	2,77	2,34	43
Ф -II	1,23	2,77	2,02	75
Ф-III	1,14	2,86	1,95	91

figures given above shows that with an increase in the time of extraction the amount of condensed fractions in the lignin increases more than twofold. Similar calculations cannot be performed in a series of natural lignins of the early vegetation periods, since in DLA-I, DLA-II, and DLA-K it is impossible to neglect the amount of p-coumaryl units and, in addition, pyrocatechol structures have been found in them [2].

The β-vinyl and benzyl ester protons in the C_3 side chain appear in region II. In this zone at about 4 ppm, in the spectra of the three F fractions a signal appears clearly that apparently corresponds to benzyl acetate groups ArCH(OAc)—C—C [7]. The number of protons in this region is a maximum in F-I and changes in the following sequence: F-III<F-II<F-I>DLA-K> DLA-II>DLA-I. Consequently, in nonacetylated lignins the amount of benzyl alcohol groups changes in the lignin preparations in the same sequence as in the above-mentioned series.

The protons present in coumarane structures appear in zone III. It follows from Table 1 that the amount of such is a maximum in the DLA of the early vegetation periods and decreases in the DLA of the ripe stems. With an increase in the time of extraction the amount of coumarane structures in fractions F-II and F-III also decreases.

Region IV includes methoxy groups and the α-, β-, and γ-protons of the C_3 side chain. For the different preparations, the zone of the methoxy protons is between 6.0 and 6.8 ppm. The numbers of α-, β-, and γ-protons were calculated by deducting the signal of the methoxyl proton from the total signal and vary in different lignin preparations from 1.62 to 3.44 protons per C_9 unit in DLA-K and F-II, respectively. Calculation of the numbers of protons attached to the C_3 chains (regions II, III, and IV) also shows that these numbers are different. Consequently, in preparations of DLA of the stem the degrees of substitution of the side chain are not the same. The smallest number of aliphatic protons and, consequently, the highest degree of substitution are found in the DLA-K and F-III. Unfortunately, it is impossible from these facts to judge the degree of condensation of the lignins through the side chains, since the protons in them are replaced by several types of bonds: C—O—C, C=O, C—C, C—OH. According to the biogenetic theory of lignin, there should be about four protons in the C_3 side chain [4]. As can be seen from Table 1, this rule is observed for DLA-I, DLA-II, and F-I.

In region V are the protons of aromatic acetoxy groups, and in VI the protons of aliphatic acetoxy groups. Consequently, in these regions it is possible to calculate the acetoxy protons and, therefore, the numbers of phenolic and aliphatic hydroxyls. From the numbers of protons in these regions it follows that in DLA-I and DLA-K there is about one phenolic group per C_9 unit. In DLA-II there are 0.60 and in F-I 0.32. These values are not in bad agreement with the analytical results if one takes into account the fact that part of the aromatic acetate groups present in the ortho position to the biphenyl bond gives a signal in zone VI. This leads to a low result in a determination of phenolic OH groups from PMR spectra.

The numbers of aliphatic OH groups in DLA-I and F-I are about 1, and in DLA-II and DLA-K they are more than 1 (1.30 and 1.50, respectively), which is in harmony with the analytical results. In analyzing the number of hydroxyls, it may be noted that the DLAs of the early vegetation periods are more highly hydroxylated than the DLA of the ripe stems, the largest number of OH groups being present in the DLA-K.

Region VII is due to the signals of highly screened protons, possibly belonging to aliphatic methyl and methylene groups. In the DLA of the early vegetation periods and the bolls their numbers range from 0.90 (DLA-K) to 2.28 (DLA-I) and fall sharply to F-I (0.36). The protons giving signals in this region are obviously attached to methine carbon atoms, provided that these groups are not directly bound to oxygen-containing groups.

Thus, a study of lignins with the aid of PMR spectroscopy has confirmed the results of analyses and has given additional information on the functional groups and the nature of the structural units, particularly in relation to the degree of condensation of the DLA preparations.

EXPERIMENTAL

The dioxane lignin preparations were acetylated for 24 h with a tenfold amount of an equimolar mixture of acetic anhydride and pyridine at room temperature. The acetylated lignins were precipitated in water and, after drying, were purified by reprecipitation from dioxane solutions in ether. The preparations obtained were carefully dried in a vacuum desiccator over P_2O_5.

All the PMR spectra were taken on a JNM-4H-100/100 MHz spectrometer at T_{room} = 22-24°C, c 10-12% by weight, 10 — HMDS, τ-scale, solvent deuterochloroform.

SUMMARY

On the basis of an analysis of the PMR spectra of cotton-plant dioxane lignins collected in various vegetation periods, it has been found that they have different degrees of substitution of the C_3 side chain and different degrees of condensation through the aromatic nuclei. The most highly condensed is the dioxane lignin of the early vegetation period, and the least, the DLA of the ripe stems.

It has been established that with an increase in the time of extraction, condensation takes place through the aromatic nuclei.

It has been shown that the number of α-alcoholic groups in the dioxane lignins studied are different.

LITERATURE CITED

1. C. H. Ludwig, in: Lignins, K. V. Sarkanen and C. H. Ludwig (eds.), Interscience, New York (1971), pp. 399-344.
2. N. A. Veksler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prir. Soedin., 573 (1976).
3. N. A. Veksler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prir. Soedin., 100 (1977).
4. N. A. Veksler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prir. Soedin., 122 (1978).
5. C. H. Ludwig, B. J. Nist, and J. L. McCarthy, J. Am. Chem. Soc., 86, 1186, 1196 (1964).
6. A. G. Zhuravlev, Yu. V. Gladkov, I. V. Sen'ko, and V. M. Reznikov, in: The Chemistry and Uses of Lignins [in Russian], Riga (1974), p. 149.
7. C. Simionescu and A. Cernatescu-Asandei, Celul. Hirtie, 21, No. 6, 308 (1972).
8. D. E. Bland and S. Sternhell, Austr. J. Chem., 18, 401 (1956).
9. M. S. Rakhmatullaev, N. A. Versler, L. S. Smirnova, and Kh. A. Abduazimov, Khim. Prir. Soedin., 82 (1979).

A STUDY OF THE STRUCTURE OF LIGNINS OF HEALTHY AND WILT-AFFECTED COTTON PLANTS OF THE VARIETY TASHKENT-1

B. Kh. Pulatov and Kh. A. Abduazimov

UDC 547.458.84+549.927.2

Continuing a study of the dioxane lignins of healthy and wilt-affected cotton plants of variety Tashkent-1 (DLCT) according to vegetation period, we have performed nitrobenzene oxidation and cleavage with sodium in liquid ammonia of the natural and isolated dioxane lignins. It has been established that in the wilt-affected samples of cotton-plant stems the amount of guaiacyl and syringyl structural units increases.

In a preceding communication [1] we described the isolation and characteristics of the dioxane lignins (DLs) of healthy cotton plants of the variety Tashkent-1 and of plants affected by wilt (*Verticillium dahliae* Kleb.) according to vegetation period. Continuing an investigation of the lignins isolated from stems of the early period (DLCT-I, 7-8 cotyledons)

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 392-396, May-June, 1979. Original article submitted December 19, 1978.

0009-3130/79/1503-0341$07.50