SUMMARY

1. A method has been developed for isolating urease from watermelon seeds which includes the stages of extraction, fractionation with ammonium sulfate, precipitation with ethanol, and subsequent lyophilization. A sample of enzyme has been obtained with a specific activity of 140 μmole of urea per 1 minute per 1 mg of protein.

2. It has been shown by gel filtration on Sepharose 4B that the sample contains a component possessing urease activity and characterized by a molecular mass of 480,000.

3. The dependence of the urease activity on the pH and the kinetic parameters of the enzyme has been studied.

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A STUDY OF THE LIGNIN OF THE SEA ISLAND COTTON PLANT OF VARIETY S-6030

M. K. Mirzakhmedova, L. S. Smirnova, and Kh. A. Abduazimov

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The dioxane lignin has been isolated by Pepper's method with a yield of 9.5% on the Komarov lignin from the ripe stems of the sea island cotton plant of variety S-6030. The developed emperical formula has been calculated, the IR and UV spectra have been taken, and the molecular-mass distribution has been studied. The dioxane lignin is polydisperse. $\bar{M}_w$ = 10,100, $\bar{M}_n$ = 5350.

Continuing a study of cotton-plant lignin, we have investigated the dioxane lignin (DLA) obtained from the stems of the sea island cotton plant *Gossipium barbadense* collected after the gathering of the crop, S-6030.

The DLA was obtained by Pepper's method [1] with a yield of 9.5% on the Komarov lignin from the comminuted stems of the cotton plant that had been extracted with ethanol-benzene and washed with hot water. The DLA was purified by two reprecipitations by pouring aqueous dioxane (1:9) solutions of it into absolute ether.

On the basis of elementary analysis and functional-group analysis [2] taking carbohydrates into account [3], the developed empirical formula was calculated:

molecular mass 204.58.

$$C_9H_{8,7}O_{0,59}(OCH_3)_{1,13}(OH_{phe})_{0,29}(OH_{al})_{1,19}(O_{CO})_{0,32}(O_{ar-al})_{0,71}.$$

It can be seen from the formula that the DLA of the sea island cotton plant contains a high level of methoxy groups. On an average, to each phenylpropane structural unit (PPSU) there is one methoxy group and another two to each 5 or 6 PPSUs. The low content of phenolic

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OH groups is unusual. In DLAs studied previously from medium-fiber varieties of the cotton plant it is considerably higher: 0.4-0.7 OH_{Phe} per one PPSU [4, 5].

The C_3 side chain is highly hydroxylated: in each PPSU there is one hydroxyl, and another two hydroxyl groups for each five PPSUs. A carbonyl group is present in each third PPSU.

The UV spectrum of the DLA is characteristic for lignin and has a maximum at 280 nm and a minimum at 250 nm.

In the IR spectrum of the DLA there are two bands characteristic of lignins. Below we give the characteristics of the IR spectrum calculated by the method of Karklin' and Erin'sh [6]:

Frequency cm^{-1}	ROD	Interpretation of the absorption bands
3455	0.89	Stretching vibrations of O—H bonds of hydroxyls and hydrogen bonds
2940	0.70	Stretching vibrations of C—H bonds and CH_2 and CH_3 groups
2850	0.55	Stretching vibrations of C—H in CH_2 and CH_3 in OCH_3 groups
1730	0.51	Stretching vibrations of carbonyl groups
1665	0.56	
1600	0.88	Stretching vibrations of double bonds in aromatic rings
1510	1.00	
1470	1.02	Deformation vibrations of C—H bonds in OCH_3 groups
1430	0.89	
1330	0.87	
1275	1.23	Stretching vibrations of C—O—C bonds in OCH_3 groups
1230	1.20	Stretching vibrations of C—O—C bonds in ethers
1130	1.81	Wagging vibrations of CH_3 groups
1040	1.07	Symmetrical stretching vibrations of C—O—C bonds in OCH_3 groups.

The results of a study of the molecular mass distribution of the DLA by the column gel chromatography method showed that it was polydisperse. The calculation of $\bar{M}_w$ and $\bar{M}_n$ using G-75 gel, dimethyl sulfoxide as solvent and eluent, and the coefficients calculated previously [7] showed that the DLA had a low molecular mass: $\bar{M}_w$ = 10,100, $\bar{M}_n$ = 5350, $\bar{M}_w/\bar{M}_n$ = 1.89.

EXPERIMENTAL

Preparation of the DLA. Air-dry cotton plant stems (50 g) that had been comminuted (0.25 mm), extracted with ethanol—benzene (1:1) for 48 h, and washed three times with hot water were extracted at 90°C in a current of nitrogen with stirring with 900 ml of dioxane, 80 ml of water, and 18 ml of concentration hydrochloric acid for 0.5 h. The extract was separated off and the plant material was washed with aqueous dioxane (1:9) which was combined with the main extract, and after neutralization with sodium bicarbonate this was evaporated in vacuum to a volume of 50-70 ml. The concentrated extract was added in drops to cold water acidified with HCl to pH 1-2. The DLA that precipitated was separated off by centrifugation washed with water, and dried over P_2O_5. Yield 1.3 g. Elementary composition: C — 60.02%; H — 5.61%.

Functional-group Analysis [2] (%): Methoxy groups — 17.76 (determined by the Zeisel-Viebeck—Schwappach method); carbonyl groups — 4.35 (by the oximation method); total hydroxy groups — 12.7 (by acetylation); phenolic hydroxyls — 2.42 (by the chemosorption method); carbohydrates bound to the lignin — 2.11 (determined by Bertrand's method [3] after hydrolysis of the DLA with 5% sulfuric acid).

The UV spectrum was taken on a SF-26 spectrophotometer in aqueous dioxane (1:9), and the IR spectrum on a UR-20 instrument in tablets with potassium bromide.

Gel chromatography was carried out on a column 1.2 × 45 cm filled with G-75 gel in DMSO. V_e = 31 ml, V_o = 9.8 ml in terms of dextran blue with a molecular mass of 2,000,000. The coefficients found previously [7] were used to calculate molecular masses.

SUMMARY

The dioxane lignin has been isolated from the ripe stems of the sea island cotton plant of variety S-6030, a developed semiempirical formula has been derived for it, its UV and IR spectra have been recorded, and its molecular mass has been determined (10,100).

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INSECT PHEROMONES AND THEIR ANALOGS.

VIII. SYNTHESIS OF THE (Z) AND (E) ISOMERS OF 2-METHYLOCTADEC-7-ENE AND OF 2-METHYL-7,8-EPOXYOCTADECANE

V. N. Odinokov, G. G. Balezina,
U. M. Dzhemilev, G. Yu. Ishmuratov,
D. V. Amirkhanov, V. P. Krivonogov,
F. Kh. Sitnikova, and G. A. Tolstikov

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A highly stereospecific method for the synthesis of racemic (Z)-disparlure has been developed which is based on the reduction of 2-methyloctadec-7-yne with the aid of 9-borabicyclo[3.3.1]nonane and the epoxidation of the resulting (Z)-2-methyloctadec-7-ene with p-methoxycarbonylperbenzoic acid. The ^{13}C NMR spectra of the (Z) and (E) isomers of 2-methyloctadec-7-ene and 2-methyl-7,8-epoxyoctadecane, which unambiguously confirm the structures of these compounds, are given. It has been established that (E)-2-methyloctadec-7-ene exhibits a moderate attractant activity while the (Z) isomer does not attract the gypsy moth. The addition of 5-25% of (E)-disparlure increases the biological activity of (Z)-disparlure.

In the synthesis of racemic (Z)-disparlure by the Witting olefin-forming reaction [1, 2], or by the hydrogenation of 2-methyloctadec-7-yne [3-6], from 2 to 15% of the (E) isomer is formed. Only a few methods permit d,l-disparlure exclusively of the (Z) configuration to be obtained [7-9]. Information on the influence of (Z)-2-methyloctadec-7-ene and (E)-2-methyl-7,8-epoxyoctadecane as impurities on the biological activity of the sex pheromone of the gypsy moth (*Lymantria dispar*), (Z)-2-methyl-7,8-epoxyoctadecane is inadequate. It is known, in particular, the (Z)-2-methyloctadec-7-ene is an inhibitor of the sex pheromone [10], while conversely, (E)-2-methyl-7,8-epoxyoctadecane in amounts not exceeding 10-15% enhances the attractant properties of (Z)-d,l-disparlure [11]. There is no information whatever on the action of (E)-2-methyloctadecene on the behavior of gypsy moth males.

Institute of Chemistry, Bashkir Branch, Academy of Sciences of the USSR, Ufa.

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