in the maturing cotton boll. Our data clearly demonstrate that this is not the sole mechanism for the formation of cellulose-C^{14} in the cotton boll and show that at least a part of the original radioactive sugar enters the structure of the polysaccharide through its scission products. In fact, the exchange of label between the terminal positions of D-glucose could be due to the reversible isomerization of dihydroxyacetone-1-C^{14} 1-phosphate to D-glyceraldose-3-C^{14} 3-phosphate by the enzyme triose isomerase,[12] in the glycolytic process[13] for the breakdown of D-glucose. This process could account for the conversion of a substantial amount of the radioactive sugar into other constituents of the cotton boll, such as the seed oil; these points will be treated in a succeeding communication.

In early spring, when a longer period of time was required for the development and maturity of the cotton bolls, a single experiment (B, Table I) furnished only a 4.7% radiochemical yield of cellulose with the label distribution shown, demonstrating a more extensive breakdown and resynthesis of the D-glucose-1-C^{14} before its conversion into cellulose. It appears that the period of maximum cellulose formation which, according to Greathouse,[5] should be 21 days after fertilization of the flower, varies with the change in the rate of development of the cotton bolls due to such factors as the season of the year and the general condition of the plants in the greenhouse. This could explain the difference between the yield and distribution of the label in our experiments (A, B and C). It would be logical to assume that should the labeled D-glucose be introduced before the period of maximum cellulose formation, the sugar would have a better chance to participate in other complex functions of the cotton boll.

Experimental

Preparation of C^{14}-Labeled Cotton Cellulose.—A number of cotton plants, *Gossypium hirsutum* var. *Paula*, were cultivated in 7-inch pots in the greenhouse of the Botany Department of The Ohio State University, and the dates of their flowerings[14] were carefully recorded. After some preliminary experiments (including expt. B of Table I) to find the best conditions, 15.7 μc. of D-glucose-1-C^{14} (150 mg., prepared according to Isbell and co-workers[15]) was introduced in equal quantities into 10 well developed 21-day old cotton bolls according to the method of Greathouse.[5] This experiment was performed during the month of July (expt. A, Table I). The plant was first segregated and not watered for 1 day. Then 1 cm. of the stem immediately below the boll was thinly sliced upward and the cut portion was introduced into a small glass cup of 1-ml. capacity sealed to a glass rod held in a clamp. The cup contained a solution of 15 mg. of D-glucose-1-C^{14} in 3 drops of water. After complete absorption of the sugar solution by the plant, the cup was washed twice with 2 drops of water and the washings were allowed to enter the plant in the same manner. This process was normally over within a few hours. The sliced stem was then carefully taped and the plant was watered and allowed to continue its normal course of development. The treated boll matured after 40 to 50 days. The resulting radioactive cotton was separated from the seeds and the dried capsule and its radioactivity was roughly counted at infinite thickness. It was then mixed with other crops, dewaxed and purified according to Greathouse[5]; yield 9.19 g. of dry cellulose (1.672 μc.).

During September another 10 selected 21-day old cotton bolls, growing in a more suitable environment (Expt. C, Table I), were treated with 33.0 μc. of D-glucose-6-C^{14} (150 mg.[16]). They matured after 30 to 35 days; yield 9.77 g. of purified, dry radioactive cellulose (7.784 μc.).

Hydrolysis of Cellulose-C^{14}.—The purified radioactive cellulose from each experiment (Table I) was divided into 400-mg. portions which were radioassayed at infinite thickness and found to have about the same degree of activity. One gram from each experiment was then selected as a representative sample and was hydrolyzed with 72% sulfuric acid by the method of Monier-Williams.[8] The hydrolyzate was neutralized with a solution of barium hydroxide, centrifuged and filtered. The filtrate was passed through a column containing 40 ml. of an equal mixture of Amberlite resins IR-120 (H) and IR-4B (OH)[17] and concentrated under reduced pressure to 10 ml. In order to separate the partially hydrolyzed materials, the concentrated solution was passed through another column containing 20 g. of an equal mixture of activated carbon and Celite[18] according to Whistler and Durso,[9] and washed with 200 ml. of water. The solution was then lyophilized and the remaining sirup was crystallized from methanol and 2-propanol; yield 0.64 g. of D-glucose-C^{14}.

Radioactivity Determination.—D-Glucose-C^{14} from the hydrolysis of radioactive cellulose was oxidized to potassium D-gluconate by the procedure of Moore and Link[10] and counted after recrystallization from methanol and water. The distribution of label within the anhydro-D-glucose unit of the cellulose-C^{14} was obtained by degradation of the potassium D-gluconate with sodium periodate according to the method of Eisenberg,[11] and further oxidation of the resulting formic acid and formaldehyde to carbon dioxide. Thus the carbon dioxide from positions 1, 6 and 2–5 (combined) of the cellulose anhydro-D-glucose units was converted to barium carbonate and assayed for radioactivity (counted).

Counting Methods.—All samples were counted as solids at infinite thickness using a mica window Geiger tube[19] connected to a decade scaler,[20] and compared with a standard of the same material. The potassium D-gluconate used as a reference was standardized by conversion to barium carbonate[11] which was in turn compared with standard barium carbonate obtained from Tracerlab.[21] The samples were counted long enough to reduce the random counting error to $\pm 3\%$, and the background radiation was kept to a minimum with a 2 in. thick lead shield.

Acknowledgment.—We wish to express our gratitude to Dr. Glenn A. Greathouse for his kindness in supplying us with detailed information on the plant-dosing techniques employed, and to Professor Bernard S. Meyer, of the Department of Botany, for the use of a greenhouse.

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(17) Products of the Rohm and Haas Co., Resinous Products Division, Philadelphia 5, Pa.

(18) A product of the Johns-Manville Co., New York, N. Y.

(19) Thyrode 1B67/VG-10A, Victoreen Instrument Co., Cleveland 3, Ohio.

(20) Potter Instrument Co., Inc., Flushing, N. Y.

(21) Tracerlab, Inc., Boston 10, Mass.

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The *para*-Claisen Rearrangement. II. The α- and γ-Methylallyl Ethers of Methyl *o*-Cresotinate[1,2]

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Earlier work[3] on the preparation of the α-ethyl-

(1) Taken from the Ph.D. dissertation of Robert L. Crecelius, University of Wyoming, 1954.

(2) This work was supported by a Frederick Gardner Cottrell Grant from the Research Corporation.

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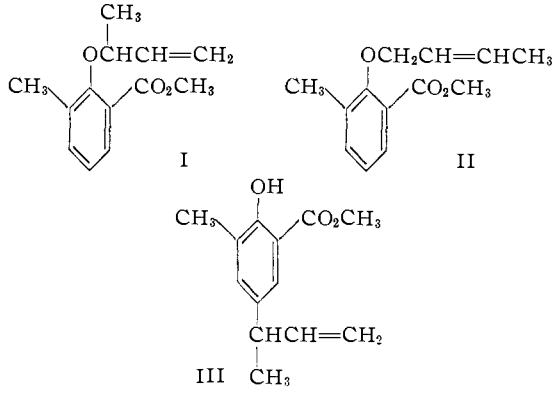
allyl ether of methyl *o*-cresotinate failed to furnish a method for separating the thermally sensitive α-ether from the isomeric γ-ethylallyl ether which formed concurrently and preponderantly. Since uncontaminated α-substituted allylic ethers of this series were desired for rearrangement rate studies, it seemed worthwhile to investigate the methyl homolog in the hope that the smaller steric requirement of the methyl group might permit the formation of the α-methylallyl ether in quantities more favorable to its isolation in pure form. Accordingly, the preparations of the α- and γ-methylallyl ethers of methyl *o*-cresotinate (I and II) were undertaken by methods already described.[3]



Our experience with these preparations parallels exactly that of Rhoads, Raulins and Reynolds with the ethyl homologs. The preparation and the rearrangement of II proceed smoothly to give the expected products in good yield and in agreement with the report of Mumm and Moller.[4] The preparation of I, however, is characterized by a multiplicity of products among which have been found I and II, their corresponding rearrangement products III and IV, and higher boiling diallylated material.

Evidence for the presence of I in the low-boiling ethereal fraction of the reaction mixture is furnished by ozonolysis data, infrared spectral analysis and a preferential rearrangement[3] of the mixture of I and II to yield the hitherto unreported 2-carbomethoxy-4-(α-methylallyl)-6-methylphenol (III). The structure of the latter is established by ozonolysis and infrared data and its non-identity with the rearrangement product obtained from II.

The rearrangement of both ethers, then, appears to involve mainly, if not exclusively, a non-inverting process. Such results would be expected in view of recent reports on the *para*-rearrangement.[3,5] Details of the preparation and characterization of the compounds involved in this work are recorded in the Experimental section. The heterogeneity of the reaction mixture encountered in the preparation of I discouraged further efforts to secure pure α-ether samples in this series.

It may be noted that the preferential rearrangement of this pair of isomeric ethers did not proceed so cleanly as did that of the ethyl homologs.[3] Apparently, the difference in the rates of rearrangement of the α- and γ-isomers is considerably smaller in the present case.

Experimental[6]

Materials.—The isomeric methylallyl chlorides were prepared from methylvinylcarbinol[7] by the method of Kepner, Winstein and Young.[8] Properties of the pure chlorides: α-methylallyl chloride, b.p. 56.2–56.5° at 585 mm., $n^{20}D$ 1.4152; γ-methylallyl chloride, b.p. 76.4–76.6° at 585 mm., $n^{20}D$ 1.4354.

The preparation of methyl *o*-cresotinate was carried out as described previously[3] but with a longer reflux period of 33 hours. In this manner, a yield of 72% was realized, b.p. 145–146° at 54 mm., $n^{20}D$ 1.5345.

Preparation of γ-Methylallyl 2-Carbomethoxy-6-methylphenyl Ether (II).—The reaction was carried out according to the method of Mumm and Moller[4] except that Claisen alkali was substituted for the sodium hydroxide extractions for removal of phenolic material. A yield of 66% of pure ether was realized, b.p. 94.5–95.5° at 0.3 mm., $n^{20}D$ 1.5178, ferric chloride test negative.

Methanolic potassium hydroxide hydrolysis of a sample of this material gave the corresponding acid in 76% yield, m.p. 74.4–75.2° from hexane (reported 76°[4]).

Rearrangement of II.—The ether was rearranged in refluxing diethylaniline. After three hours, the reaction mixture was worked up according to the method of Rhoads, Raulins and Reynolds[3] to give a 73% yield of 2-carbomethoxy-4-(γ-methylallyl)-6-methylphenol (IV), colorless oil, b.p. 127–129.5° at 1.7 mm., $n^{20}D$ 1.5351, ferric chloride test dark blue. Hydrolysis furnished the corresponding acid, 2-carboxy-4-(γ-methylallyl)-6-methylphenol (V), m.p. 133.4–134.6° (reported 135–136.5°[4]), fine colorless needles from hexane. Hydrogenation of the unsaturated acid over Adams catalyst proceeded with the absorption of 1 mole equivalent of hydrogen. Crystallized from hexane, the hydrogenated acid, **2-carboxy-4-butyl-6-methylphenol** (VI), melted at 108.0–108.6°.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.2; H, 7.8. Found: C, 69.2; H, 8.0.

Reaction of Methyl *o*-Cresotinate with α-Methylallyl Chloride.—The reaction was carried out using the sodium methoxide–methanol method described earlier.[3] A 30% excess of α-methylallyl chloride was used and the reaction mixture was refluxed 39 hours. The products were worked up in the usual manner,[3] Claisen alkali being employed to separate the phenolic and ethereal fractions.

Phenolic Fraction.—The phenolic fraction was separated by fractionation *in vacuo* to yield 23% of the original methyl *o*-cresotinate as a forerun followed by arbitrarily cut fractions boiling over the range 166–182° at 16 mm. with refractive indices of $n^{20}D$ 1.5324 to 1.5350. The higher boiling phenolic fractions accounted for an additional 36% of the starting materials.

Identification of the components of the phenolic fractions was made by hydrolysis to the corresponding acids. Hydrolysis of the low boiling forerun yielded cresotinic acid. Hydrolysis of the high boiling end fractions, b.p. 176–182° at 16 mm., gave 90–94% yields of V, m.p. 133.5–135.8°. Hydrolysis of the first cut of the high boiling phenolic material (immediately following the methyl *o*-cresotinate forerun), b.p. 166–171.5° at 16 mm., gave a crude acid, m.p. 79.2–85.2°, indicative of a mixture. Repeated recrystallization of this acid from hexane raised the m.p. to 92.4–93.3°, undepressed when mixed with authentic 2-carboxy-4-(α-methylallyl)-6-methylphenol (VII) (*vide infra*).

Ethereal Fraction.—The ethereal material was separated into low and high boiling fractions by rapid distillation *in vacuo*. The low boiling ethereal material distilled over the range 82.5–97° at 0.3 mm., and showed a refractive index increase from $n^{20}D$ 1.5107 to $n^{20}D$ 1.5185. Material collected in this range accounted for 22% of the starting materials and gave no ferric chloride test. The high boiling ether fraction (8% of theoretical yield, calculated as diallylated material) distilled from 110 to 129° at 0.25 mm., and gave a negative ferric chloride test.

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(6) All melting points are corrected. Microanalyses were performed by the Clark Microanalytical Laboratory, Urbana, Ill.

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Hydrolysis of the first cut of the low boiling ethereal material gave a pale yellow oil (86%) which, after trituration with hexane at −70°, formed colorless needles melting when warmed to room temperature.

Hydrolysis of the last cut of low boiling ethereal material (b.p. 94–97° at 0.3 mm.) produced colorless needles (71%) of γ-methylallyl 2-carboxy-6-methylphenyl ether, m.p. 74.5–75°, identical with an authentic sample by mixed m.p.

Hydrogenation of a sample of the high boiling ethereal fraction proceeded with the uptake of 3 mole equivalents of hydrogen. Hydrolysis of the hydrogenated product yielded VI, thereby identifying the high boiling ethereal material as diallylated substance(s) (*cf.* ref. 3).

Preferential Rearrangement.—A sample of the low boiling ethereal material from the reaction of α-methylallyl chloride and methyl *o*-cresotinate was subjected to a preferential rearrangement at 120° (see ref. 3 for procedure). After 27 hours a control on the pure γ-methylallyl ether indicated that it had suffered 10% rearrangement and showed a blue-green ferric chloride test while the ethereal mixture showed a deep royal blue test. The refractive index of the mixture changed from $n^{20}D$ 1.5145 to $n^{20}D$ 1.5184 during the 27-hour period while that of the control sample changed from $n^{20}D$ 1.5178 to 1.5195. The preferentially rearranged material was worked up in the usual manner and on distillation gave a 23% yield of **2-carbomethoxy-4-(α-methylallyl)-6-methylphenol** (III), colorless oil, b.p. 97–103° at 0.9 mm., $n^{20}D$ 1.5196, ferric chloride test deep blue.

Hydrolysis of a sample of III gave 97% of a crude solid which after repeated recrystallization from hexane formed colorless needles of **2-carboxy-4-(α-methylallyl)-6-methylphenol** (VII), m.p. 93–94.3°. Admixture with pure V gave a m.p. of marked depression. A mixed m.p. with the lower melting acid obtained from the phenolic fractions showed no depression (*vide supra*).

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.9; H, 6.8. Found: C, 69.6; H, 6.7.

Microhydrogenation of VII over Adams catalyst proceeded with the uptake of 1 mole equivalent of hydrogen to form **2-carboxy-4-(2-butyl)-6-methylphenol** melting, after recrystallization and sublimation, at 94.4–95.1°. Admixed with authentic VII it melted at 89–90°.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.2; H, 7.8. Found: C, 69.4; H, 7.9.

Ozonolysis.—The apparatus and procedure used for ozonolysis were those previously described.[3] Results are shown in Table I.

TABLE I

Sample	H_2CO found as dimethone deriv., %	Terminal methylene cpd., %
Allyl ether of methyl *o*-cresotinate (model cpd. for ethers)	56 ± 5.0	100
γ-Methylallyl ether (II)	0	0
Fract. 1 of the low boiling ethereal (mixt. of I and II)	31.2	55.3 ± 5
2-Carbomethoxy-4-allyl-6-methylphenol (model cpd. for phenols)	55.2 ± 0.3	100
2-Carbomethoxy-4-(γ-methylallyl)-6-methylphenol (IV)	0	0
2-Carbomethoxy-4-(α-methylallyl)-6-methylphenol (III)	37.5	67.9 ± 0.5

Infrared Spectra.[9]—Infrared spectra were obtained in the 2–15μ region with a Perkin–Elmer spectrophotometer, model 12C. Characteristic peaks in the significant 9–11.5μ region are shown in Table II.

The strong absorption bands at 10.86μ in the ether mixture from the α-ether preparation and at 10.87μ in the preferentially rearranged material confirm the presence of the α-methylallyl compounds. The absence of detectable absorption at 10.36μ in the spectrum of III affirms the preferential nature of the rearrangement of the mixture of I and II and strongly suggests that the main course in the rearrangement of I is a non-inverting one.

TABLE II

II	10.33μ (str)
Fract. 1 of low boiling ethereal (mixt. of I and II	10.06–10.30μ (mod str); 10.86μ (str) (broad absorption)
IV	10.36μ (str)
III	10.08μ (mod str); 10.87μ (str)

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(9) For a discussion of the characteristic infrared absorption associated with terminal methylene groups and internal double bonds in these compounds see ref. 3. Complete spectra for the compounds reported in this paper are contained in the Ph.D. dissertation of R. L. Crecelius, obtainable through Interlibrary Loan from the University of Wyoming Library.

A Transhydrogenase and Reduced Triphosphopyridinenucleotide Involved in the Oxidation of Desoxycorticosterone to Corticosterone by Adrenal Tissue[1]

BY MAX L. SWEAT AND MARION D. LIPSCOMB

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Since the observation of Hayano, Dorfman and Prins[2] that adrenal slices and homogenates were capable of converting desoxycorticosterone to a substance with glycogenic activity, several laboratories have studied steroid conversions in adrenal a-cellular preparations. McGinty, *et al.*,[3] demonstrated the conversion of 17-hydroxy-11-desoxycorticosterone to 17-hydroxycorticosterone in beef adrenal homogenates. Sweat[4] was able to effect this conversion in a mitochondrial fraction and on the basis of heat, rate and inhibitor studies concluded that the conversion was enzymic in nature. He further pointed out that the reaction would not proceed if the fumarate moiety of the Hayano–Dorfman buffer medium were replaced with phosphate buffer. Recently, Hayano and Dorfman[5] have observed that oxidized TPN restores the activity of washed or aged preparations of adrenal tissue. Various laboratories[6–9] have reported that metabolites other than fumarate enhance the oxidation of 11-desoxysteroids. In this report we wish to present evidence that one of the cofactors in the conversion of desoxycorticosterone to corticosterone is reduced triphosphopyridinenucleotide and that one of the enzymes involved in generating this cofactor in adrenal tissue is a transhydrogenase.

Mitochondria equivalent to 40 mg. of adrenal tissue were suspended in 1 ml. of an incubation medium containing 0.04 *M* sodium phosphate buffer (*p*H 7.4), 0.004 *M* $MgCl_2$, 40 μg. of desoxycorticosterone and one or a combination of the following cosubstrates and cofactors: 0.01 *M* fumarate, malate, citrate, *cis*-aconitate, isocitrate

(1) This investigation was supported by a research grant (A-331) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

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