[A CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Constitution of the Galactomannan of the Seeds of the Kentucky Coffee Bean (*Gymnocladus dioica*)[1]

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The galactomannan of the seeds of the Kentucky coffee bean is composed of 80% D-mannose and 20% D-galactose approximately. The methylated galactomannan gives upon hydrolysis 2,3,4,6-tetra-*O*-methyl-D-galactose (1 mole prop.), 2,3,6-tri-*O*-methyl-D-mannose (3 mole props.), 2,3-di-*O*-methyl-D-mannose (1 mole prop.) and a small amount (3.3%) of 2,3,4,6-tetra-*O*-methyl-D-mannose. The structural significance of these findings and those from periodate oxidation of the polysaccharide is discussed.

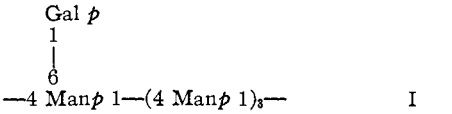
In previous communications constitutional studies on the galactomannan of carob gum[2] and of guar gum[3] have been reported. This paper is concerned with the constitution of the galactomannan polysaccharide, extracted from the seeds of the Kentucky coffee bean, *Gymnocladus dioica*, as revealed principally by methylation studies.

The polysaccharide was isolated by extracting the hard vitreous inner coating of the seeds with alkali. Obtained directly or after purification by acetylation and regeneration from the acetate, the polysaccharide was a snow-white amorphous powder. It formed viscous solutions in water and in alkali which gave a precipitate with Fehling solution but did not reduce it. Solutions of the galactomannan were gelled by adding small amounts of borax. The polysaccharide was found to be composed of D-galactose (identified as the methyl phenylhydrazone) and D-mannose (identified as the phenylhydrazone) in the proportions 1 to 4, respectively, a result in reasonably good agreement with previous findings.[4,5]

Methylation of the polysaccharide with methyl sulfate and alkali afforded the corresponding methyl derivative $[\alpha]D \pm 0°$ (acetone) which appeared from fractional precipitation data to be essentially homogeneous.

Upon hydrolysis the methylated galactomannan gave rise to the following cleavage products which were separated by column and paper chromatography: (a) 2,3,4,6-tetra-*O*-methyl-D-galactose (1 mole prop.), characterized as the anilide; (b) 2,3,6-tri-*O*-methyl-D-mannose (3 mole props.), characterized by bromine oxidation to the crystalline γ-lactone; and (c) 2,3-di-*O*-methyl-D-mannose (1 mole prop.), characterized by oxidation with nitric acid and isolation of the bismethylamide of dimethyl *meso*-tartaric acid. In addition there was isolated 3.3% of 2,3,4,6-tetra-*O*-methyl-D-mannose which was identified as the crystalline anilide.

These findings show that all the D-galactose residues occupy terminal positions in the molecule and that for each single galactose unit there are four D-mannose residues, one of which affords the 2,3-di-*O*-methyl derivative and hence must represent a branching point in the molecule, being linked through positions 1, 4 and 6; the other three mannose units occupy positions in a chain of residues joined by 1,4-glycosidic bonds. This evidence, together with the stability of the polysaccharide and its methyl derivative to hydrolytic cleavage suggesting that all the residues are of the pyranose type, point to a formulation such as I for the average repeating unit of the molecular complex.



I

It will be apparent that some of the mannose units that give rise to the 2,3,6-tri-*O*-methyl derivative might well be interposed between the mannose residue at which branching occurs and the non-reducing terminal units.

The presence of 3.3% of 2,3,4,6-tetra-*O*-methyl-D-mannose in the hydrolysate of the methylated galactomannan could arise from a non-reducing terminal unit of a complex of 25 mannose units to which are attached 5 galactose non-reducing terminal units. Such a formulation representing the whole molecule is considered unlikely since the galactomannan is non-reducing to Fehling solution whereas a relatively small polysaccharide molecule would be expected to display reducing properties as is the case with laminarin which is composed of about 20 glucose residues.[6]

The non-reducing character of the galactomannan could be explained by a cyclic structure but the highly viscous character of its solutions does not favor any molecular structure comprising a relatively small number of sugar residues.

A more likely structural possibility is that it is the average repeating unit of the galactomannan which is composed of about 30 sugar residues (25 of mannose and 5 of galactose); of these all of the galactose residues are located at terminal positions and there is also one terminal unit of mannose.

Support for this general type of formulation was forthcoming from the fact that the Kentucky coffee bean galactomannan was found to consume 6 moles of periodate for every 5 sugar residues with the liberation of one molecular proportion of formic acid, the latter arising from the terminal non-reducing residues. Moreover, the periodate oxidized polysaccharide was found to contain no intact residues of either mannose or galactose.

Inasmuch as the Kentucky coffee bean poly-

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(2) F. Smith, THIS JOURNAL, **70**, 3249 (1948).

(3) M. C. Rafique and F. Smith, *ibid.*, **72**, 4634 (1950).

(4) L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, **16**, 28 (1944).

(5) E. Anderson, *Ind. Eng. Chem.*, **41**, 2887 (1949).

(6) J. J. Connell, E. L. Hirst and E. G. V. Percival, *J. Chem. Soc.*, 3494 (1950).

saccharide shows a relatively low rotation ([α]D + 29°, in water) as does its acetate ([α]D + 20°, in acetone) and methyl ether ([α]D ± 0°, in acetone), it is believed that most of the glycosidic bonds joining the mannose units of the polysaccharide are of the β-type. The terminal units of galactose may be joined by 1,6-α-glycosidic bonds as is the case with guar galactomannan,[7] and carob bean galactomannan.[8]

This galactomannan conforms to the general structural pattern already established for other galactomannans that have been examined in detail, for all of them in the form of their methyl derivatives give rise to the same cleavage fragments, namely, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,-6-tri-*O*-methyl- and 2,3-di-*O*-methyl-D-mannose.[2,3,9-11] The main difference between the various galactomannans is probably to be traced to the length of the side chains and the frequency at which branching occurs along the main chain of mannose units.[12]

Experimental

Isolation of the Galactomannan.—The ripe seeds were removed from the bean pods collected from trees in St. Paul, Minnesota in October, 1949. The seeds were split open with a hammer and the endosperm discarded. The polysaccharide material is found firmly attached to the inner side of the seed coat as a tough, clear, vitreous layer. The seed coats were soaked in water overnight to allow the polysaccharide to swell to a colorless jelly-like mass which could readily be removed with a spatula. The portions of gel were digested on a hot water-bath with 3–4 parts of *N* sodium hydroxide until complete dispersion was achieved. The viscous extract was filtered through a brass screen (200 mesh), acidified with acetic acid and poured into an equal volume of methanol. The crude galactomannan so obtained was centrifuged, redissolved in *N* sodium hydroxide and the solution, after neutralization with acetic acid, poured with stirring into an equal volume of methanol. The precipitate was centrifuged, washed successively with methanol, acetone, petroleum ether and dried *in vacuo*. The galactomannan showed $[\alpha]^{23}D$ +22° in *N* sodium hydroxide (*c* 1.0). Aqueous solutions of the polysaccharide gelled upon adding borax and gave a copper complex upon adding Fehling solution.

Hydrolysis of the Galactomannan.—When a portion of the galactomannan was refluxed with *N* sulfuric acid for 11 hr., the solution acquired a constant specific rotation, $[\alpha]^{22}D$ +30.4°. The solution was neutralized with barium carbonate, filtered and evaporated *in vacuo* to a sirup which showed $[\alpha]^{23}D$ +28.5° (calcd. for a mixture of 4 parts of D-mannose and 1 part D-galactose, [α]D +28°).

Identification of D-Mannose and D-Galactose.—Treatment of a portion of the hydrolysate with 20% phenylhydrazine in ethanol containing 3% acetic acid for several days at 5° yielded D-mannose phenylhydrazone, m.p. and mixed m.p. 189°.

Another portion of the hydrolysate treated with 20% methylphenylhydrazine in ethanol containing 3% acetic acid for 2–3 days at 5° afforded D-galactose methylphenylhydrazone, m.p. and mixed m.p. 173°.

Chromatographic Analysis of the Galactomannan Hydrolysate. (a) Qualitative.—Paper chromatography using 1-butanol–ethanol–water (4:1:5) showed the presence of two components, the R_F values of which corresponded to mannose and galactose.

(b) Quantitative.[13]—0.1 ml. of a solution of the hydrolysate (0.2521 g.) in methanol (10 ml.) was placed on Whatman No. 1 filter paper (22″ × 8″) and the sugars separated by irrigation for 70 hours with 1-butanol–ethanol–water. The component sugars, located by spraying marginal strips with Tollens reagent, were each extracted from the unsprayed central portion of the chromatogram with water (50 ml.). In triplicate determinations one ml. of each extract (after filtration through glass wool) was mixed with 1 ml. of 20% aqueous phenol and 5 ml. of concd. sulfuric acid. A blank experiment was carried out at the same time on a piece of paper cut from the irrigated portion of chromatogram containing no sugar. The intensity of color formed was determined by a Junior Coleman spectrophotometer and the concentration of sugar ascertained by reference to standard curves for D-galactose and D-mannose. The results of duplicate analyses showed D-mannose, 77.8, 79.4 and D-galactose 22.2, 20.6. This corresponded to approximately 4 parts of D-mannose and 1 part of D-galactose.

Acetylation of the Galactomannan.—The crude polysaccharide (3 g.) was dispersed in formamide (200 ml.) by shaking at room temperature for 2 days. Pyridine (30 ml.) was added followed by acetic anhydride (25 ml.), the latter being added dropwise during 1 hour. After keeping overnight, the viscous brownish reaction mixture was poured with stirring into water. The white fibrous material was filtered, washed with water until the washings were colorless and dissolved in acetone (100 ml.). The acetone solution was poured into water whereupon all the colored impurity in the acetate passed into the water. The snow-white galactomannan acetate was filtered, washed with water, alcohol, petroleum ether and dried, yield 3.65 g., $[\alpha]^{22}D$ +20° in acetone (*c* 1.0).

Regeneration of the Galactomannan from the Acetate.—A solution of the acetate (2.5 g.) in acetone (50 ml.) was refluxed in the presence of 45% KOH (50 ml.). The upper acetone layer lost its viscous character as deacetylation proceeded and the polysaccharide passed into the lower alkaline layer. At the same time the lower layer became very viscous. This lower layer was separated by decantation of the upper acetone layer and poured with stirring into methyl alcohol. The mixture was acidified with glacial acetic acid and the polysaccharide was separated (centrifuge), dissolved in water (cold) and reprecipitated with methanol. A further reprecipitation in the same manner afforded the purified galactomannan which was dried by solvent exchange as above, 1.1 g., $[\alpha]^{22}D$ +29° in water (*c* 0.5).

Oxidation of the Galactomannan with Sodium Periodate.—When the oxidation was carried out according to a procedure used previously,[14] the results given in Table I were obtained. An average repeating unit, composed of 4 mannose units joined by 1,4-bonds and 1 galactose side-chain attached to position 6 of one of the mannose units, would consume 6 moles of periodate with the liberation of 1 mole of formic acid.

TABLE I

Expt. no.	Periodate consumed/ anhydrohexose unit	No. hexose units giving 1 mole HCOOH
1	1.22	5.05
2	1.2	4.9
3	1.0	5.2
4	1.26	5.4
5	Not determined	5.0[a]

[a] Determined by the potassium periodate method.[15]

Examination of Periodate Oxidized Kentucky Coffee Bean Galactomannan.—The periodate oxidized compound, which remained undissolved in the periodate oxidation reaction (in one experiment) was filtered off, washed with water and dried.

A sample (20 mg.) of it was dissolved in 0.1 *N* H_2SO_4 (1 ml.) and heated for 12 hours on the boiling water-bath. The solution, brownish yellow in color, was neutralized with barium carbonate filtered and evaporated to dryness. Chromatographic analysis using 1-butanol–ethanol–water (4:1:5) failed to reveal any residual galactose or mannose in the hydrolysate.

Methylation of the Galactomannan.—To a solution of the polysaccharide (10 g.) in 30% sodium hydroxide (400 ml.),

(7) R. L. Whistler and P. F. Durso, THIS JOURNAL, **73**, 4189 (1951).
(8) B. W. Lew and R. A. Gortner, *Arch. Biochem.*, **1**, 325 (1943).
(9) J. W. Swanson, THIS JOURNAL, **71**, 1510 (1949).
(10) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948).
(11) Z. F. Ahmed and R. L. Whistler, THIS JOURNAL, **72**, 2524 (1950).
(12) K. J. Palmer and M. Ballantyne, *ibid.*, **72**, 736 (1950).
(13) M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Nature*, **168**, 167 (1951).
(14) M. Abdel Akher and F. Smith, THIS JOURNAL, **73**, 994 (1951).
(15) T. G. Halsall, E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1399 (1947).

methyl sulfate (200 ml.) and 30% sodium hydroxide (200 ml.) were added dropwise with vigorous stirring during 1.5 hours. No external heat was applied and no attempt was made to cool the reaction mixture which developed heat. After 4 hr., the reaction mixture was heated on a water-bath (50°) while methyl sulfate (100 ml.) and 30% sodium hydroxide (300 ml.) were added dropwise during 1 hr. The reaction mixture was then heated on the boiling water-bath for 20 minutes. Since the methylated polysaccharide did not separate at this stage the methylation mixture was neutralized with 5 *N* sulfuric acid and dialyzed in cellophane tubing against running tap-water for 1–2 days to remove most of the inorganic salts. The dialyzed solution was made alkaline and concentrated *in vacuo* to a small volume. The residue containing the partially methylated polysaccharide was remethylated at 60° with methyl sulfate (100 ml.) and 30% sodium hydroxide (300 ml.), the reagents being added dropwise during 2 hr. The reaction mixture was heated on the boiling water-bath, cooled, neutralized and dialyzed. The product was isolated by evaporation *in vacuo* and subjected to a third methylation as before with methyl sulfate (150 ml.) and 30% sodium hydroxide (450 ml.) at 50–55°, acetone being added when necessary to keep the methylated product in solution. Upon neutralization with sulfuric acid the methylated product separated as a heavy sirup. The methylated galactomannan was extracted three times with 1-butanol (100 ml.) and the combined butanol extracts were concentrated *in vacuo* to a thick sirup which was dissolved in acetone (100 ml.) and subjected to two further methylations with methyl sulfate (100 ml.) and 30% sodium hydroxide (300 ml.) at 55° in the usual way. After the final methylation the methylated galactomannan separated as an amorphous pale yellow product when the methylation mixture was heated on the boiling water-bath to expel the acetone. The fully methylated galactomannan isolated by extraction with butanol and evaporation, was dissolved in chloroform; the chloroform extract was dried ($MgSO_4$) filtered and freed from solvent by evaporation *in vacuo* (yield 6 g.).

The crude methylated galactomannan was dissolved in acetone and a series of fractions obtained by precipitation with increasing amounts of ether. Each fraction was dissolved in acetone and the solution poured with stirring into petroleum ether. The fractions obtained in this way were amorphous almost white powders. Their properties are recorded in Table II.

TABLE II

FRACTIONAL PRECIPITATION OF THE METHYLATED GALACTOMANNAN OF KENTUCKY COFFEE BEAN

Fraction	1	2	3	4	5
Wt., g.	2.4	0.7	0.1	0.4	0.5
OMe, %	43.3	45.1	45.2	45.2	45.0
$[\alpha]^{23}D$ (acetone, *c* 1)	±0°	±0°	±0°	±0°	±0°
η_{sp} 21.5° (*c* 0.45 in *m*-cresol)	0.236	0.236	....	0.228	0.234

Hydrolysis of the Methylated Galactomannan.—A 25-mg. sample of each of the 5 fractions of the methylated polysaccharide was heated for 12 hr. in a sealed tube with *N* hydrochloric acid (1 ml.). The solutions were neutralized (Ag_2CO_3) filtered and evaporated *in vacuo* to dryness.

When each of the hydrolysates was examined by paper chromatography using methyl ethyl ketone–water azeotrope as the solvent and *p*-anisidine as the spray reagent three components were detected. Their R_F value, 0.55, 0.67 and 0.80 corresponded to those of 2,3-di-*O*-methyl-D-mannose (0.55), 2,3,6-tri-*O*-methyl-D-mannose (0.65) and 2,3,4,6-tetra-*O*-methyl-D-galactose (0.80). When higher concentrations of the hydrolysate were tested, a component moving faster (R_F, 0.85) than the tetra-*O*-methyl-D-galactose was detected. This was later proved to be 2,3,4,6-tetra-*O*-methyl-D-mannose (see below).

In larger scale experiments the methylated polysaccharide (1.0 g.) was hydrolyzed for 5 hours with *N* sulfuric acid (25 ml.) on a boiling water-bath when the rotation became constant ($[\alpha]^{23}D$ +12.7°). The solution was neutralized with barium carbonate, digested on the hot water-bath and filtered. The combined filtrate and washings were concentrated to a sirup which was freed from inorganic impurities by extraction with acetone (yield 0.96 g.).

Column Chromatographic Separation of the Hydrolysate of Methylated Galactomannan.—A number of experiments were carried out on approximately 0.5 g. of the hydrolysate using methyl ethyl ketone–water azeotrope as the irrigating solvent and an automatic fraction collector in the manner previously described.[16]

A typical result of an examination of the eluate from the column is shown in Table III while the quantitative result is given in Table IV.

TABLE III

COLUMN CHROMATOGRAPHIC ANALYSIS OF THE HYDROLYSATE OF METHYLATED KENTUCKY COFFEE BEAN GALACTOMANNAN. EXAMINATION OF THE ELUATE

Tube no.	Component	Methyl sugar
13–18	1	2,3,4,6-Tetra-*O*-methyl-D-mannose
19–35	2	2,3,4,6-Tetra-*O*-methyl-D-galactose
36–72	3	2,3,6-Tri-*O*-methyl-D-mannose
117–150	4	2,3-Di-*O*-methyl-D-mannose

TABLE IV

COLUMN CHROMATOGRAPHIC ANALYSIS OF THE HYDROLYSATE (0.54 G.) OF METHYLATED KENTUCKY COFFEE BEAN GALACTOMANNAN[a]

Component	Wt., g.	$[\alpha]D$ (H_2O)	Mole ratio
(1) 2,3,4,6-Tetra-*O*-methyl-D-mannose	0.019	+ 33.5°	(0.17)
(2) 2,3,4,6-Tetra-*O*-methyl-D-galactose	.108	+112°	1
(3) 2,3,6-Tri-*O*-methyl-D-mannose	.284	− 7°	2.8
(4) 2,3-Di-*O*-methyl-D-mannose	.116	− 13°	1.2
Total wt. recovered 0.527 g.			
Recovery = 97.5%			

[a] In a duplicate experiment using the same amount of hydrolysate (0.54 g.) the results of the fractionation were as follows: Component (1), 0.019; (2), 0.113 g.; (3). 0.282 g.; (4), 0.118 g. These values corresponded to the same molecular ratios as those recorded in Table IV.

Component (1), 2,3,4,6-tetra-*O*-methyl-D-mannose, was shown to be contaminated with small amounts of component 2 (2,3,4,6-tetra-*O*-methyl-D-galactose). On the assumption that it is pure and represents the terminal non-reducing end in the polysaccharide, it may be deduced that the average repeating galactomannan molecule is composed of about 30 hexose residues 6 of which are D-galactose and 24 are D-mannose and that the hydrolysate of the methylated polysaccharide contains 2,3,4,6-tetra-*O*-methyl-D-mannose (1 mole prop.), 2,3,4,6-tetra-*O*-methyl-D-galactose (6 mole props.), 2,3,6-tri-*O*-methyl-D-mannose (16 mole props.), and 2,3-di-*O*-methyl-D-mannose (7 mole props. approx.).

Quantitative Paper Chromatography of the Hydrolysate of the Methylated Galactomannan.—A quantitative separation was carried out on paper in the usual way and the components determined by means of the phenol–sulfuric acid method.[13] In duplicate experiments the mole ratios for 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose were found to be: 1·0 (1·0), 3·07 (3·0) and 1·1 (1·02), respectively.

It is believed that this result is more reliable than that obtained by column chromatography since the separation of the components on paper is better than it is on the column and, furthermore, material extracted from the cellulose is taken into account in the analysis by paper chromatography.

Identification of the Components of the Hydrolysate of the Methylated Galactomannan. (1) **2,3,4,6-Tetra-*O*-methyl-D-mannose.**—When component 1 of Table III was treated for 2 hours with boiling ethanol containing aniline (1.2 moles) there was obtained upon removal of solvent

(16) L. A. Boggs, L. S. Cuendet, M. Dubois and F. Smith, *Anal. Chem.*, **24**, 1148 (1952).

2,3,4,6-tetra-*O*-methyl-D-mannose anilide m.p. and mixed m.p. 141° (after recrystallization from petroleum ether).[17]

(2) **2,3,4,6-Tetra-*O*-methyl-D-galactose.**—Treatment of a portion (50 mg.) of component 2, Table III, with aniline in alcohol, as under "1" above, afforded 2,3,4,6-tetra-*O*-methyl-D-galactose anilide which had m.p. and mixed m.p. 195°, $[\alpha]^{23}D$ −137° in pyridine (*c* 0.5) (after recrystallization from ethanol).[18]

(3) **2,3,6-Tri-*O*-methyl-D-mannose.**—Component 3, (0.5 g.) (see Table III), was oxidized with bromine at room temperature for 7 days. Isolation of the lactone in the usual way yielded 2,3,6-tri-*O*-methyl-D-mannono-γ-lactone, m.p. and mixed m.p. 81°, $[\alpha]^{24}D$ +61° initial value in water (*c* 1.0), after recrystallization from ether.[2]

(4) **2,3-Di-*O*-methyl-D-mannose.**—Component 4, (0.3 g.), was oxidized with nitric acid (sp. gr. 1.42) in the usual way. After esterification of the product with ethereal diazomethane, removal of solvent and distillation *in vacuo*, crystalline methyl dimethyl *meso*-tartrate was obtained and this yielded the crystalline bismethylamide m.p. and mixed m.p. 212°.[19]

(17) J. C. Irvine and D. McNicoll, *J. Chem. Soc.*, **97**, 1452 (1910).

(18) W. N. Haworth, J. V. Loach and C. W. Long, *ibid.*, 3146 (1927).

(19) W. N. Haworth and D. I. Jones, *ibid.*, 2349 (1927).

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[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE[1]]

Application of Tosylate Reductions and Molecular Rotations to the Stereochemistry of Lignans[2]

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The *trans*-(2,3)-*trans*-(3,4) arrangement of α-conidendrin (III, R = H) has been confirmed by chemical evidence. Comparison of molecular rotations shows that the configuration at C_2 and C_3 of α-conidendrin, and hence of (+)-isolariciresinol (II, R = H, R' = OH) and (−)-matairesinol (I, R = H), is identical with that of podophyllotoxin (IV, R = H, R' = OH, R'' = CH_3) and related compounds. In turn, the configurations of these lignans have been correlated with those of (−)-guaiaretic acid (XIII, R = H) and of galbulin (II, R = CH_3, R' = H) by the reduction of the tosylates XIV (R = CH_3, R' = OTs) and II (R = CH_3, R' = OTs) to the corresponding methyl compounds (R' = H).

Significant progress has been made over the years in attempts to elucidate the relative configurations at the different asymmetric centers of lignans[3] and to interrelate stereochemically the various members of this class of compounds. Thus Haworth has demonstrated the *trans*-(2,3) arrangement of (−)-matairesinol (I, R = H)[4a,5] and shown that this relationship persists without any configurational change at C_2 and C_3 in (+)-isolariciresinol (II, R = H, R' = OH)[4b] and hence in α-conidendrin (III, R = H).[5,6] Cyclization of diarylbutanes (I) to phenyltetralins (II, III) introduces a third asymmetric center (at C_4), and Haworth postulated a *trans*-(3,4) relation in the naturally-occurring lignans of this type, on the assumption that the most stable isomer should be formed during ring-closure.[7] Since the lariciresinol–isolariciresinol rearrangement[8] certainly proceeds by a carbonium ion mechanism, (+)-isolariciresinol, and therefore α-conidendrin, should be expected to possess the stable *trans*-(3,4) configuration in accordance with Haworth's postulate. A more direct proof, however, still appeared desirable. Moreover, Haworth's assumption does not necessarily apply to ring-closures proceeding under biologic conditions, and indeed it was shown that a group of naturally-occurring phenyltetralins, endowed with activity against experimental tumors (namely, podophyllotoxin (IV, R = H, R' = OH, R'' = CH_3), desoxypodophyllotoxin (IV, R = R' = H, R'' = CH_3),[9] demethylpodophyllotoxin (IV, R = R'' = H, R' = OH),[10] α-peltatin (IV, R = OH, R' = R'' = H),[11] and β-peltatin (IV, R = OH, R' = H, R'' = CH_3)[11]), possess a *trans*-(2,3)-*cis*-(3,4) configuration.[12]

It has now become possible to interrelate the configurations of these biologically active lignans with those of α-conidendrin and (−)-matairesinol. A stereospecific series of reactions led from α-conidendrin dimethyl ether (III, R = CH_3) to α-retrodendrin dimethyl ether (V) with retention of configuration at all asymmetric centers.[13] α-Retrodendrin dimethyl ether fails to undergo base-catalyzed epimerization at C_3,[13] characteristic of the *trans*-(2,3)-*cis*-(3,4) lactones IV.[12] However, we have found that refluxing with sodium acetate in methanol converts it to methyl α-retrodendrate dimethyl ether (VI), a reaction which parallels the

(1) National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare.

(2) Presented in part before the Medicinal Chemistry Division of the American Chemical Society in New York City, September 15, 1954.

(3) R. D. Haworth, *Nature*, **147**, 255 (1941).

(4) (a) R. D. Haworth and D. Woodcock, *J. Chem. Soc.*, 154 (1939); (b) 1054 (1939).

(5) (a) R. D. Haworth and L. Wilson, *ibid.*, 71 (1950). (b) The "*trans*" and "*cis*" configurations of matairesinol and isomatairesinol,[5a] respectively, are designated with reference to the lactone ring and correspond to a (−) and a *meso* configuration in the open-chain diols[5a] derived from these compounds. Similarly, the substituents at C_2 and C_3 in (+)-lariciresinol dimethyl ether[4b] are *cis* with respect to the tetrahydrofuran ring, while they are *trans* (with respect to the tetralin ring) in the isolariciresinol dimethyl ether (II, R = CH_3, R' = OH) derived from it. This change does not represent a true Walden inversion, but is produced by the difference in the reference ring systems and is more clearly visualized by examination of models. Hydrogenolysis of (+)-lariciresinol dimethyl ether[4b] and reduction of (−)-matairesinol dimethyl ether[5a] yield the same (−)-diol (XIV, R = CH_3, R' = OH). In compounds like α-conidendrin (III, R = H), the *trans*-(2,3) arrangement refers to both the tetralin and the lactone rings.

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(8) R. D. Haworth and W. Kelly, *ibid.*, 384 (1937).

(9) J. L. Hartwell, A. W. Schrecker and J. M. Johnson, THIS JOURNAL, **75**, 2138 (1953).

(10) M. V. Nadkarni, J. L. Hartwell, P. B. Maury and J. Leiter, *ibid.*, **75**, 1308 (1953).

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