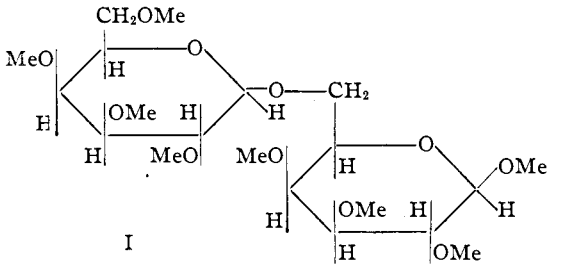
[CONTRIBUTION FROM THE WOOD CONVERSION LABORATORY OF THE UNIVERSITY OF IDAHO]

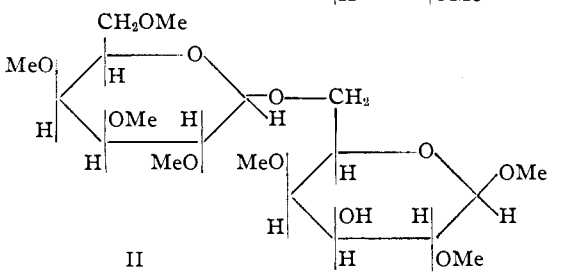
The Constitution of Arabo-galactan. III. The Location of the Arabinose Component

BY E. V. WHITE

It has been shown that the water-soluble gum[1] of the western larch, *Larix occidentalis*, yields the glycosides of 2,4-dimethyl-*d*-galactose, 2,3,4-trimethyl-*d*-galactose, 2,3,4,6-tetramethyl-*d*-galactose, and 2,3,5-trimethyl-*l*-arabinose in the approximate molecular ratio 3:1:2:1 upon alcoholysis of the methyl ether derivative.[2] Furthermore, by partial methanolysis of arabo-galactan methyl ether,[3] two methylated disaccharides have been obtained in crystalline form. These are, respectively, octamethyl-6-*d*-galactosidogalactose (I)and heptamethyl-6-*d*-galactosidogalactose (II).[3a]



I



II

Apparently the terminal galactose anhydride units of the polysaccharide are engaged by oxygen linkage through the 1 position to the 6 position of adjacent galactose residues and the question arises as to the mode of union of the terminal arabofuranose unit.

As is well known, the furanopentosides are considerably more susceptible to acid hydrolysis than are the corresponding derivatives of the pyranopentoses and especially the pyranohexoses, although, under similar conditions, the rate of hydrolysis is a function of the particular saccharide under consideration. Advantage has been taken of this phenomenon in the investigation of certain oligosaccharides,[4a,b] xylan[5] and arabic acid.[6] Hirst and co-workers[7] report its successful application to arabo-galactan but do not give details of the experiment.

The relative rates of hydrolysis of the galactopyranosides as compared with those of the corresponding arabofuranosides under similar conditions are not known, although it is indicated indirectly that the difference is not as large as might be expected. Thus, since the methyl arabopyranosides are hydrolyzed about 1.5 times as rapidly as the methyl galactopyranosides[8] and since the only known methyl arabofuranoside is hydrolyzed about 10 times as rapidly as the corresponding pyranoside,[9] it is to be expected that the arabofuranosides would hydrolyze about 15 times as rapidly as the galactopyranosides. In the case of arabo-galactan, therefore, wherein six molecules of galactose are associated with one residue of arabofuranose, hydrolysis of the furanopentose unit should be accompanied theoretically by concomitant hydrolysis of 0.4 unit of galactose.

The strictly preferential hydrolysis of the arabofuranose component of arabo-galactan is thus a matter of some difficulty. However, after mild treatment, any substantial change in the ratio of the components isolated upon alcoholysis of a partially hydrolyzed, fully methylated material as compared with those obtained upon similar treatment of the methyl ether derivative would indicate the method of linkage of the pentose unit.

With these considerations in mind, a quantity of arabo-galactan was separated from larch sawdust and divided into two portions. One of these was subjected to partial hydrolysis. Samples of the hydrolyzing solution were removed at intervals and analyzed for residual polysaccharide. The non-hydrolyzed pentose fraction of the latter was then determined by the Tollens method. The

(1) Schorger and Smith, *Ind. Eng. Chem.*, **8**, 494 (1916).
(2) White, THIS JOURNAL, **63**, 2871 (1941).
(3) White, *ibid.*, **64**, 302 (1942).
(3a) In Part II the structures (I) and (II) are incorrectly represented in the relative location of H and OMe at position 4 of the monosaccharide units involved.
(4) (a) Bourguelot and co-workers, *Comp. rend.*, **126**, 280 (1898); **132**, 571 (1901); (b) Kuhn and Grundherr, *Ber.*, **59**, 1655 (1926).
(5) Hirst and Peat, *J. Chem. Soc.*, 1983 (1937).
(6) Smith, *ibid.*, 744 (1939).
(7) Hirst, Jones and Campbell, *Nature*, **147**, 25 (1941).
(8) Isbell and Frush, *J. Research N.B.S.*, **24**, 125 (1940).
(9) Montgomery and Hudson, THIS JOURNAL, **59**, 992 (1937).

TABLE I

PARTIAL HYDROLYSIS OF ARABO-GALACTAN

Acidity, 0.020 N H_2SO_4; temp., 90°

Time, hours	% Residual polysaccharide	% Arabinose in residual polysaccharide
0	100	13.6
7	94.2	9.91
16	90.0	7.24
23	88.4	6.16

results obtained, given in Table I, are represented graphically in Fig. 1.

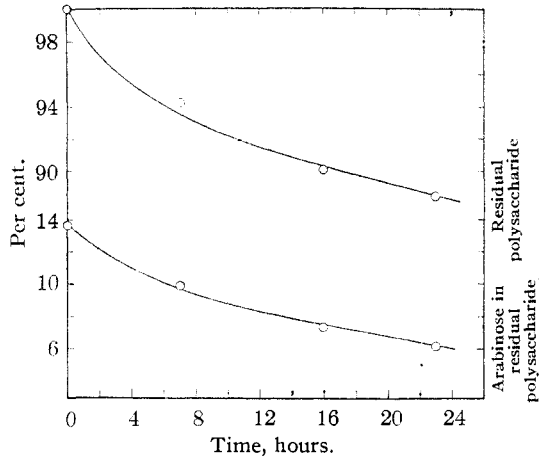


Fig. 1.—The hydrolysis of arabo-galactan (acidity, 0.02 N H_2SO_4; temp., 90°).

Hydrolysis of the gum obviously proceeds with gradual decrease in the pentose fraction of the residual polysaccharide and apparently tends toward a final value. Furthermore, within the range investigated, the decrease in yield of residual polysaccharide parallels approximately the loss of pentose, indicating that simultaneous galactose fission was relatively slight under the conditions employed.[10] No attempt was made to completely remove the arabinose component lest prolonged treatment promote undue galactose hydrolysis. The hydrolyzate, therefore, is to be regarded as a mixture of unchanged arabo-galactan and arabinose-free arabo-galactan together with some more extensively hydrolyzed material. The partially hydrolyzed product was methylated with dimethyl sulfate and alkali and subjected to simultaneous complete hydrolysis and glycoside formation with methanolic hydrogen chloride. The resulting sirup was distilled fractionally and the yield of the components compared with those obtained from the second portion of arabo-galactan which was methylated directly and subjected to alcoholysis.

An analysis of the results obtained is given in Table II as taken from Tables III and IV, respectively.

An examination of this table shows a sharp decrease in the amount of dimethyl-galactoside obtained from the glycosidic sirup of the partially hydrolyzed, fully methylated product as compared with that obtained from the ether of the original polysaccharide. Correspondingly, an increase is noted in the yield of trimethyl-galactoside. When correction is made in the glycosidic sirup for those components resulting from unchanged arabo-galactan in the partially hydrolyzed product, a substantially equimolecular ratio is obtained for the di-, tri- and tetramethyl-galactoside components[7] derived from methylated, arabinose-free arabo-galactan. The conclusion is reached, therefore, that the arabinose fraction of the polysaccharide is joined by oxygen linkage to an already di-linked galactose residue and that the new hydroxyl group formed by hydrolysis of the furanopentose residue and substituted in subsequent methylation contributes to the increased proportion of the trimethylated component in the glycosidic sirup from the partially hydrolyzed, fully methylated product.

The location of the new hydroxyl group, and therefore of the arabinose residue, was revealed by an examination of the trimethyl galactoside component of the glycosidic sirup. Thus, while arabo-galactan methyl ether yields only 2,3,4-trimethyl-methyl-galactoside as trimethyl component upon methanolysis, the partially hydrolyzed, methylated and hydrolyzed material provides a trimethyl galactoside fraction which furnishes 2,3,4-trimethyl-galactose and 2,4,6-trimethyl-galactose. The latter evidently originates through methylation of a hydroxyl group in the 6-position of a galactose residue formed during partial hydrolysis by removal of the arabinose unit. The same galactose residue normally occurs in the alcoholysis products of arabo-galactan methyl ether as 2,4-dimethyl-methyl-galactoside. In the original arabo-galactan, therefore, the arabinose

(10) After twenty-three hours of treatment, arabinose removed by hydrolysis = 13.6 − (6.16 × 88.4/100) = 8.2%; residual galactan = (88.4 − 5.4) = 83.0%; hydrolysis ratio galactose : arabinose = $\left(\frac{8.2}{13.6} \div \frac{86.4 - 83.0}{86.4} \times \frac{180}{160}\right)$ = 13.6:1. The experimental value approximates the theoretical hydrolysis ratio 15:1 and indicates 4.5% galactose fission under conditions hydrolyzing 62% of the arabinose component. Similar calculations made after sixteen and seven hours of hydrolysis yield the ratios 13.8:1 and 16.2:1, respectively. However, these evaluations of hydrolysis ratio must be regarded as approximations since galactan hydrolysis may occur leaving an alcohol insoluble residue without formation of galactose.

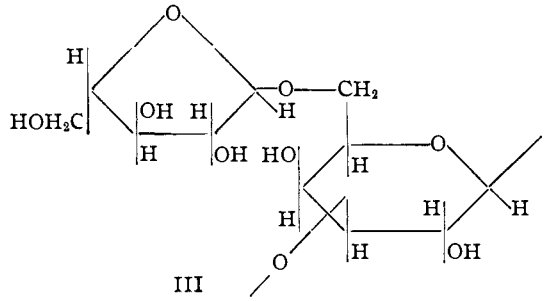
TABLE II

GLYCOSIDIC SIRUP ANALYSIS

	From arabo-galactan Me ether			From partially hydrolyzed, fully methylated arabo-galactan				
	Grams	Total yield %	Mol. ratio	Grams	Total yield %	Unchanged[b] arabo-galactan, grams	Arabinose-free arabo-galactan Grams	Mol. ratio
Trimethyl-methyl-arabinoside	9.6[a]	12.9	1.00	3.6[b]	5.9	3.6	0	0
Tetramethyl-methyl-galactoside	21.2	28.2	1.82	20.2	33.0	8.7	11.5	1.97
Trimethyl-methyl-galactoside	12.5	16.7	1.14	15.3	2 5.0	4.1	11.2	2.01
Dimethyl-methyl-galactoside	31.5	42.2	3.04	22.1	36.1	11.5	10.6	2.02
Totals	74.7	100.0	7.00	61.2	100.0	27.9	33.3	6.00

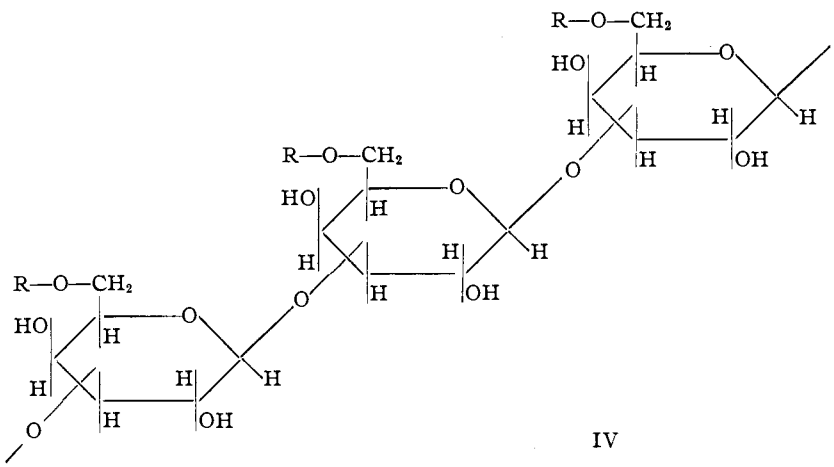
[a] Calcd. from arabinose content of arabo-galactan. [b] Calcd. from arabinose content of arabo-galactan and that of the hydrolyzed product.

fraction occurs as a 1-6 linked arabofuranosido-galactan III.



III

A consideration of these facts, together with the knowledge that octamethyl- and heptamethyl-6-*d*-galactosidogalactose have been isolated through partial methanolysis of arabo-galactan methyl ether, furnishes direct information concerning six monosaccharide units of the polysaccharide wherein six galactose residues are associated with one unit of arabinose. The remaining galactose anhydride occurs as the 2,4-dimethyl derivative in arabo-galactan methyl ether and is thus tri-linked in the original polysaccharide. The exact location of this unit is not known, although the problem resolves itself into two possibilities. Thus, the tri-linked residue may be situated in the main chain of the branched structure previously indicated,[3] whereupon the constitution of the polysaccharide is represented by a "backbone" or main chain of 1-3 linked galactose anhydride units IV each substituted in the 6 position by the radical R. The repeating unit of arabo-galactan then becomes one of three main chain units bearing the radicals R, respectively, *l*-arabinose, *d*-galactose, and 6-*d*-galactosidogalactose. In the event, however, that the final tri-linked galactose anhydride is not part of the main chain but that one or more such units are engaged in side-chain linkage, a portion or all of the radicals R become of polysaccharide character and the nature of the main chain linkage is unknown. This phase of the investigation is now being extended.



IV

Experimental

Extraction and Purification of Arabo-galactan.—Larch sawdust was extracted with water and the extract, purified by filtration through norite and Super-Cel, fractionally precipitated with ethyl alcohol.[2] The precipitate thus obtained was dissolved in water, evaporated under reduced pressure at 50° to remove residual alcohol, and divided into Parts A and B, respectively. Part A was fully methylated under nitrogen with dimethyl sulfate and alkali, as described previously.[3] Part B was subjected to partial hydrolysis.

Partial Hydrolysis of Arabo-galactan.—A number of preliminary experiments indicated that hydrolysis of arabo-galactan took place rapidly when the gum was heated in sulfuric acid solutions of greater concentration than 0.05 *N*. In more dilute solutions hydrolysis proceeded progressively slower until in 0.01 *N* acid solution only slight change was noted over long periods of time. Accordingly, Part B (72.6 g. of solid) of the arabo-galactan extract was heated at 90° on the water-bath in 750 cc. of 0.02 *N* sulfuric acid.

Samples of the hydrolyzing solution were removed at intervals, cooled, and a 25-cc. portion precipitated into excess rapidly stirred ethyl alcohol. The precipitate was washed with fresh alcohol to remove residual monosaccharide, dissolved in water, and made up to 100 cc. volume. Aliquot portions of this solution were then analyzed for total solid and for furfural distilled by the Tollens method. The results are given in Table I and are illustrated graphically in Fig. 1.

Methylation of Partially Hydrolyzed Arabo-galactan.—The hydrolyzing solution of arabo-galactan after twenty-three hours of treatment was cooled (635 cc.) and precipitated into excess rapidly stirred ethyl alcohol. The precipitate was removed from the supernatant liquor (C), washed with fresh alcohol, dissolved in water, and evaporated to a thin sirup. The latter was methylated at 25° under nitrogen using 300 cc. of methyl sulfate and 900 cc. of 30% sodium hydroxide. The reagents were added dropwise and simultaneously over a period of five hours. Acetone (200 cc.) was added over the interval to reduce foaming. After complete hydrolysis of the methyl sulfate the partially methylated product separated from the inorganic reaction components. The latter were removed and the residue remethylated under similar conditions. After four methylations, retreatment effected no increase in methoxyl content of the product, which was isolated by extraction of the methylation liquors with chloroform. The extract, dried over magnesium sulfate, decolorized with norite and filtered, was evaporated to a sirup and precipitated into excess rapidly stirred petroleum ether (30–60°). The precipitate, taken up in ether, filtered and evaporated to dryness, yielded a friable glassy solid of light yellow color; yield, 57 g. (Found: MeO, 44.7.).

Isolation and Identification of Arabinose.—The supernatant alcoholic liquor (C) was neutralized with barium carbonate, decolorized with norite, and evaporated to small volume. The resulting solution was precipitated into excess alcohol and a small quantity (1.5 g.) of residual gum removed. The solution, containing 5.6 g. of arabinose by the Tollens method, evaporated to a sirup and taken up in fresh alcohol, crystallized, yielding crude arabinose identified as the benzyl-phenylhydrazone: m. p. 174°.

Methanolysis of Fully Methylated, Partially Hydrolyzed, Arabo-galactan and of Arabo-galactan Methyl Ether.—Fifty-six grams of fully methylated, partially hydrolyzed arabo-galactan was dissolved in 360 cc. of anhydrous pure methyl alcohol containing 2% of dry hydrogen chloride. After reaction in sealed tubes[4] maintained at 115° for five and one-half hours, excess acidity was neutralized with silver carbonate. The filtered solution was decolorized with norite, evaporated to a sirup, and taken up in anhydrous ether. Filtration of the solution and evaporation of solvent gave a sirupy mixture of variously methoxylated glycosidic components; yield, 62.0 g. A similar experiment performed on the methyl ether of non-hydrolyzed arabogalactan (66.0 g.) gave a similar glycosidic sirup; yield, 75.2 g.

Examination of the Glycosidic Sirups.—The sirup obtained upon methanolysis of arabo-galactan methyl ether was distilled fractionally under high vacuum, yielding the portions indicated in Table III. No attempt was made to separate trimethyl-methyl-arabinoside from tetramethyl-methyl-galactoside, although the separation of these components from trimethyl-methyl-galactoside and the latter from dimethyl-methyl-galactoside was relatively complete. In calculation of the components, the arabinoside fraction was determined upon the basis of the 6:1 molecular ratio of galactose to arabinose in arabo-galactan, while the small intermediate portions and the trimethyl fraction were apportioned on the basis of methoxyl content.

TABLE III

FRACTIONAL DISTILLATION OF THE GLYCOSIDIC SIRUP FROM ARABO-GALACTAN METHYL ETHER

			Components			
Distillate	Grams	OMe	*a*	*b*	*c*	*d*
Fraction I	28.3	61.3	9.6	18.7		
Fraction II	3.2	55.0		2.4	0.8	
Fraction III	12.2	51.5			10.9	1.3
Fraction IV	5.8	43.4			0.8	5.0
Fraction V	25.2	41.9				25.2
Total	74.7		9.6	21.1	12.5	31.5

[a] Trimethyl-methyl-arabinoside. [b] Tetramethyl-methyl-galactoside. [c] Trimethyl-methyl-galactoside. [d] Dimethyl-methyl-galactoside.

A similar fractional distillation was performed upon the glycosidic sirup resulting from methanolysis of the partially hydrolyzed, fully methylated material. The results obtained are listed in Table IV, wherein calculation of fraction composition was again determined as previously. In this case, the arabinose component is based upon unchanged arabo-galactan in the partially hydrolyzed product as determined from the arabinose content thereof.

TABLE IV

FRACTIONAL DISTILLATION OF THE GLYCOSIDIC SIRUP FROM PARTIALLY HYDROLYZED, FULLY METHYLATED ARABO-GALACTAN

			Components			
Distillate	Grams	OMe	*a*	*b*	*c*	*d*
Fraction I	23.2	61.5	3.6	19.6		
Fraction II	2.9	54.5		0.6	2.3	
Fraction III	12.9	51.6			11.8	1.1
Fraction IV	6.7	43.8			1.2	5.5
Fraction V	15.5	41.9				15.5
Total	61.2		3.6	20.2	15.3	22.1

[a] Trimethyl-methyl-arabinoside. [b] Tetramethyl-methyl-galactoside. [c] Trimethyl-methyl-galactoside. [d] Dimethyl-methyl-galactoside.

Separation of 2,4,6-Trimethyl-galactose from 2,3,4-Trimethyl-galactose.—The distilled fraction containing the major portion of trimethylgalactoside, Fraction III, Table IV, was hydrolyzed (5.0 g.) in 50 cc. *N* sulfuric acid on the boiling water-bath for twelve hours. The product was isolated in the usual manner and distilled under high vacuum [b. p. 150° (0.1 mm.)] yielding trimethyl galactose as a sirup; yield, 4.8 g. (Found: OMe, 41.8. Calcd. for $C_9H_{18}O_6$: OMe, 41.9).

A partial separation of 2,3,4-trimethyl-galactose from 2,4,6-trimethyl-galactose can be achieved through fractional crystallization of the corresponding anilides from ether-alcohol solution. A more satisfactory method was developed through the preferential reaction of the 2,3,4-

trimethyl derivative with triphenylchloromethane. Accordingly, 2.5 g. of the sirup was treated with 3.0 g. of triphenylchloromethane in 12 cc. of pyridine solution at room temperature for two days. The reaction mixture was then triturated with a small quantity of water to dissolve pyridine hydrochloride and the solution poured into an excess of rapidly stirred ice-water. The insoluble trityl derivative settled out as a gum along with residual reactant. After standing in the icebox with occasional stirring, the solution (A) was decanted from the granular residue. The latter, washed with fresh ice-water, dissolved in acetone, dried over magnesium sulfate, decolorized with norite, filtered and evaporated to a thin sirup, deposited crystals of triphenylcarbinol. The mother liquor, upon evaporation to a sirup (0.9 g.) and treatment with aniline (0.4 g.) in absolute ethanol under reflux for three hours, crystallized upon removal of solvent. Recrystallization from absolute ethanol gave the anilide of 2,3,4-trimethyl-6-trityl-galactose; yield, 0.7 g., m. p. 152°. (Found: OMe, 17.1. Calcd. for $C_{31}H_{37}O_5N$; OMe, 17.2).

The solution (A) containing unreacted 2,4,6-trimethyl-galactose was neutralized with silver carbonate and filtered. Silver ion was removed with hydrogen sulfide, and, after filtering, decolorizing with norite, and evaporating excess solvent, a sirupy residue was obtained; yield, 1.0 g. (Found: OMe, 52.2. Calcd. for $C_9H_{18}O_6$: MeO, 52.5).

The sirup, upon treatment with aniline (0.5 g.) in the usual manner crystallized upon removal of solvent. Recrystallization from ether–alcohol solution gave 2,4,6-trimethyl-galactose anilide; yield, 0.9 g., m. p. 178°.[11] (Found: OMe, 31.4. Calcd. for $C_{15}H_{24}O_5N$: OMe, 31.4.)

Summary

1. Fractional distillation of the glycosidic sirup obtained upon methanolysis of arabo-galactan methyl ether yields three main fractions. These are, respectively, dimethyl-methyl-galactoside, trimethyl-methyl-galactoside, and a mixture of tetramethyl-methyl-galactoside and trimethyl-methyl-arabinoside.

2. Based upon the 6:1 molecular ratio of galactose to arabinose in the original polysaccharide, the molecular ratio of the glycosidic components is 3:1:2:1, respectively.

3. The arabinose component of arabo-galactan is joined to a tri-linked galactose residue.

4. The position of such linkage is through the 1 position of the arabinose component to the 6 position of the galactose residue.

(11) McCreath and Smith, *J. Chem. Soc.*, 390 (1939).

MOSCOW, IDAHO RECEIVED MARCH 4, 1942

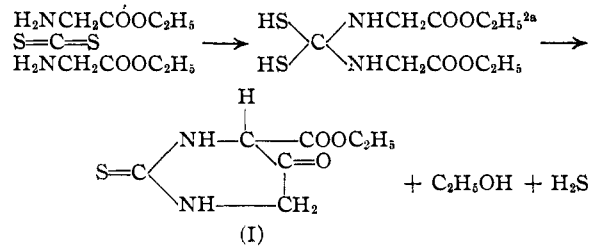
[CONTRIBUTION FROM THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

2-Thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine and Related Compounds

BY JOHN H. YOE AND GEORGE R. BOYD, JR.

Sheppard and Brigham[1] described the preparation of a heterocyclic compound which gave a deep purple colored precipitate in the presence of silver ions and suggested that it might be used as a sensitive reagent for silver. Recently Yoe and Overholser[2] have employed this compound for the colorimetric determination of silver.

The compound, 2-thio-5-keto-4-carbethoxy-1,3-dihydropyrimidine, was prepared by the action of carbon disulfide on the ethyl ester of glycine. An intermediate product was formed, diethylaminoacetate dithiocarbamate, which upon further treatment with carbon disulfide eliminated hydrogen sulfide and ethyl alcohol, closing the ring and forming the desired product. The reaction as outlined by Sheppard and Brigham[1] is as follows



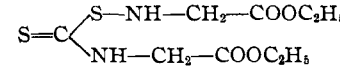
(I)

M. L. Huggins prefers to regard the structure (I) not as a closed heterocyclic ring, but as a chain which is chelated through a hydrogen bridge,[3] thus

(1) S. E. Sheppard and H. R. Brigham, THIS JOURNAL, **58**, 1046 (1936).

(2) J. H. Yoe and L. G. Overholser, *Ind. Eng. Chem., Anal. Ed.*, **14**, 148 (1942).

(2a) Sheppard and Brigham wrote the formula of this intermediate product with the following structure



Consideration of the arrangement of the electrons about the atoms, however, suggests that the structure first given is more likely.

(3) Private communication to the authors from Dr. S. E. Sheppard, Eastman Kodak Company.