A CHROMATOGRAPHIC ANALYSIS OF THE PRODUCT FROM THE TRITOSYLATION OF SUCROSE: CRYSTALLINE 6,6′-DI-*O*-TOSYLSUCROSE[1]

R. U. Lemieux and J. P. Barrette

ABSTRACT

Chromatographic analysis on silicic acid of the so-called "tri-*O*-tosylsucrose" which is formed in 95% yield on the reaction of 3 moles of *p*-toluenesulphonyl chloride with 1 mole of sucrose in pyridine at 0° has shown the substance to contain penta-, tetra-, tri-, and di-*O*-tosylsucroses in the molar ratios 0.05:0.33:1:1, respectively. Any mono-*O*-tosylsucrose present in the reaction mixture may have been lost in the isolation of the product. The composition of the product was substantially the same when the reaction was performed at −18°. The chromatogram separated the tritosylates into two subfractions. The major subfraction represented 29% by weight of the original "tri-*O*-tosylsucrose" and could be converted to 1′,2:3,6:3′,6′-trianhydrosucrose in 77.4% yield. Therefore, the so-called "tri-*O*-tosylsucrose" can contain only 25–29% of 1′,6,6′-tri-*O*-tosylsucrose. 6,6′-Di-*O*-tosylsucrose crystallized from the di-*O*-tosylsucrose fraction in 10% over-all yield.

Compton (1) obtained evidence that primary hydroxyl groups can undergo tosylation substantially more rapidly than do the secondary positions in a carbohydrate structure. He obtained yields of 41% and 36% of the 6-*O*-tosyl derivatives on monotosylations of the methyl β- and α-D-glucopyranosides, respectively. These results indicate a difference in reactivity between the primary position and the average secondary position in these compounds in the order of 7. On the other hand, Hockett and Downing (2) found that the hydroxyl groups in 1,2:5,6-di-*O*-isopropylidene-α-D-glucose and 1,2:3,4-di-*O*-isopropylidene-α-D-galactose differed in reactivity by a factor of 70. Little is known about the factors responsible for such differences in reactivity. Undoubtedly, non-bonded interactions in the transition state are important and probably mainly responsible for the generally greater reactivity of primary positions (3, 4). It should be noted, for example, that the large difference in reactivity found for the di-*O*-isopropylidene derivatives of glucose and galactose may be mainly due to the fact that the 3-hydroxyl group of the glucose derivative is eclipsed with the 4-position. Lemieux and McInnes (5) have recently shown that intramolecular hydrogen bonding also can strongly influence the rate of reaction. Furthermore, little information is available on the relative rates for the tosylation of the starting material and the first product of tosylation. Experience in related fields has shown (6, 7, 8) that this can be a dominant feature of the esterification of polyhydroxy compounds with hydrophobic reagents. Thus, when it is considered that the reactivities of both the primary and the secondary hydroxyl groups must vary considerably amongst themselves, it seems clear that attempts at preferential tosylations of the primary positions of polyhydroxy compounds can be expected to proceed in widely varying yields.

Hockett and Zief reported the preparation (9) of a substance termed "tritosylsucrose" by treatment of 1 mole of sucrose with 3 moles of tosyl chloride in pyridine at 0°. The product was not well characterized; nevertheless, it was suggested that the compound was probably "almost entirely" substituted at the three primary positions. The product is in fact highly substituted at the 6- and 6′-positions, since reaction with sodium iodide in acetone (10, 11, 12) affords material containing two iodine atoms per tosyl group. Lemieux and Barrette (13) have shown that the 1′-tosyloxy group of 1′,4,6′-tri-*O*-tosyl-

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sucrose pentaacetate is not replaced by iodine when treated with sodium iodide in acetone. Since no information was gained regarding the nature of the third tosyloxy group of the so-called "tri-*O*-tosylsucrose" and since the substance is amorphous, the results shedded no light on either the extent of substitution at the 1′-position or the distribution of the tosyl groups among the sucrose residues. The so-called "tri-*O*-tosylsucrose" was obtained in yields above 95% and analyzed well for a sucrose tritosylate. Consequently, it can be assumed that there is no appreciable tendency for a pile-up of tosyl groups on relatively few sucrose molecules. Bragg and Jones (12) have made a study of the so-called "tri-*O*-tosylsucrose" by methylation, followed by reductive detosylation and hydrolysis, and inspection of the partially methylated D-glucose and D-fructose derivatives thus formed. Inspection of their results reveals no basis for the contention that the substance "consisted mainly therefore of 6,1′,6′-tri-*O*-tosylsucrose". The present authors entertained considerable doubt as to this characterization first of all in view of ordinary kinetic considerations and also in view of the low yield (3.5%) of 1′,2:3,6:3′,6′-trianhydrosucrose which was isolated (13, 14) on alkaline alcoholysis of the substance. It was believed of real interest to obtain a proper characterization of the substance in order the better to orientate our thinking and to justify our suspicions of previous interpretations of experimental results related to this problem. Our approach was to analyze the so-called "tri-*O*-tosylsucrose" by chromatography on silicic acid columns, a procedure which Jeanloz (15) has shown to be of great value for the separation of certain carbohydrate derivatives.

"Tri-*O*-tosylsucrose" was prepared by the method of Hockett and Zief (9) both at 0° and at −18°. The amorphous products were chromatographed on paper impregnated with silicic acid (16), using diisobutyl ketone:acetic acid:water (40:25:5) as solvent. The zones were located by dipping the paper in an aqueous solution of Rhodamine 6G (16). Fluorescent spots were observed under ultraviolet light at R_f values of 0.15, 0.40, 0.65, and 0.90. It was established that these corresponded to zones of di-, tri-, tetra-, and penta-*O*-tosylsucrose, respectively, by the chromatography of the components isolated by *column* chromatography on silicic acid using the same solvent system. This paper chromatographic technique proved extremely useful for a rapid preliminary inspection of a product and the establishment of an appropriate solvent system for the column chromatography.

The column chromatograms allowed the isolation of fractions that require the "tri-*O*-tosylsucrose" prepared at 0° to be, chemically, a highly heterogeneous substance containing penta-, tetra-, tri-, and di-*O*-tosylsucroses in the molar ratios 0.05:0.33:1:1, respectively (see Table I).

TABLE I

Chromatography of "sucrose tritosylates"

	Penta-*O*-tosylsucrose	Tetra-*O*-tosylsucrose	Tri-*O*-tosylsucrose		Di-*O*-tosylsucrose	
			Fraction A	Fraction B	Fraction C*	Fraction D
Color of fluorescence	Red	Pink	Yellow	Pink	Yellow	Yellow
Tube No.	17–22	24–34	39–57	58–62	93–105	86–110
Weight of material (g)	0.18	1.53	2.65	1.17	0.92	2.11
Per cent of mixture (w/w)	2.0	17.0	29.0	13.0	10.0	24.0
Sulphur content (%)						
Found:	13.90	13.40	11.84	11.41	10.12	9.22
Calc.:	14.4	13.38	11.93	11.93	9.85	9.85
$[\alpha]_D$ (chloroform)	+42.3	+35.0	+37.5	+33.0	+54.0	+35.0

NOTE: The material, 9.05 g, was prepared at 0°.
*Crystalline 6,6′-di-*O*-tosylsucrose.

The tri-*O*-tosylsucrose fraction could be resolved into two subfractions which comprised 29.0% (tri-*O*-tosylsucrose A) and 13.0% (tri-*O*-tosylsucrose B) by weight of the original "tri-*O*-tosylsucrose". Both these fractions have thus far resisted crystallization. Their sulphur contents were in good agreement with that expected for sucrose tritosylates. That the substances were in fact different was obvious from the following properties. Whereas the A fraction gave a yellow fluorescence with Rhodamine 6G, and readily consumed 2.82 moles of periodate per mole, the B fraction produced a pink fluorescence and consumed only 1.75 moles of periodate per mole. Furthermore, the A and B fractions underwent replacement of 1.87 and 1.63 tosyloxy groups when treated with sodium iodide in acetone. These results clearly indicate that both fractions are mixtures of tritosylated sucroses. However, the high periodate consumption by the A fraction shows that it must be mainly (about 78%) 1′,6,6′-tri-*O*-tosylsucrose. This conclusion was substantiated by the formation of 1′,2:3,6:3′,6′-trianhydrosucrose (13) in 77.4% yield on treatment of the A fraction with sodium methoxide in methanol. These results provide unequivocal evidence that the trianhydrosucrose in fact arises from 1′,6,6′-tri-*O*-tosylsucrose (14).

The di-*O*-tosylsucrose fraction deposited a 10% over-all yield (by weight) of crystalline compound (fraction C), m.p. 108–110°, $[\alpha]_D$ +54° in ethanol. The sulphur content corresponded to that expected for di-*O*-tosylsucrose and the compound only reduced Fehling's solution after acid hydrolysis. The compound was acetylated and the acetyl derivative, on treating with sodium iodide in acetone at 100°, produced a substance with the iodine content expected for a diiododideoxysucrose hexaacetate. Also, the nuclear magnetic resonance spectrum of the diiodo compound was devoid of the signals characteristic for the tosyl group. These properties require that the compound be 6,6′-di-*O*-tosylsucrose. The syrup (fraction D) which remained after crystallization of the 6,6′-di-*O*-tosylsucrose had a sulphur content close to that expected for a sucrose ditosylate (see Table I). Treatment of the hexaacetate derivative with sodium iodide in acetone replaced only 1.10 of the tosyloxy groups. This result shows that the 1′-hydroxyl group and perhaps certain secondary groups can compete favorably with the 6- or 6′-hydroxyl groups, or both in the tosylation reaction. It will be seen later on that the competition is undoubtedly mainly from the 1′-position and that, consequently, the di-*O*-tosylsucrose fractions (C and D combined) may be almost entirely composed of not greatly different amounts of the 1′,6-, 1′,6′-, and 6,6′-di-*O*-tosylsucroses.

The substances isolated from the other two bands of the chromatogram possessed sulphur contents in good agreement with those expected for tetra- and penta-*O*-tosylsucroses. The results of the chromatogram are listed in Table I and the course of the tosylation is described in Fig. 1.

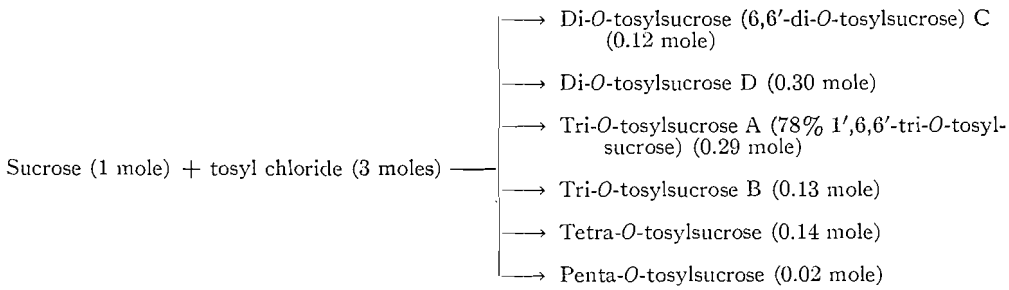

FIG. 1. Distribution of the tosylated sucroses formed in the reaction of 1 mole of sucrose with 3 moles of tosyl chloride in pyridine at 0°.

A consideration of Fig. 1 shows that the so-called "tri-*O*-tosylsucrose" does in fact contain 1′,6,6′-tri-*O*-tosylsucrose as the main component. However, the compound is only present to an extent of about 0.29 mole per mole of sucrose reacted. The fact that the compound was formed in an amount more than twice that of any other tri-*O*-tosylsucrose shows that the 1′-position is substantially more reactive than any secondary hydroxyl group. Consequently, as was discussed above, the uncharacterized di-*O*-tosylsucrose (fraction D) can be expected to consist mainly of 1′,6- and 1′,6′-di-*O*-tosylsucrose. In this respect, it is of interest to note that Hockett and Downing (2) found 2,3:4,6-di-*O*-isopropylidene-L-sorbose to undergo tosylation half as rapidly as 1,2:3,4-di-*O*-isopropylidene-D-galactose. The results clearly render it unlikely that sucrose can be acylated preferentially in high yield at the 6- and 6′-positions only. This conclusion is substantiated by the foregoing results of a ditosylation experiment. The tritylation of sucrose has been shown (17) to yield 1′,6,6′-tri-*O*-tritylsucrose pentaacetate in 45% yield. Table II describes the composition of the product obtained on a tritosylation of sucrose at −18°. It is seen that the course of the reaction was not appreciably affected by the lowering of the reaction temperature.

TABLE II

Chromatography of "sucrose tritosylate"

	Penta- and tetra-*O*-tosylsucrose	Tri-*O*-tosylsucrose		Di-*O*-tosylsucrose	
		Fraction A	Fraction B	Fraction C*	Fraction D
Weight of material (g)	2.0	3.11	1.5	0.75	2.85
Per cent of mixture (w/w)	20.0	31.1	15.0	7.5	28.5
R_f value†	0.9 and 0.65	0.40	0.40	0.15	0.15

NOTE: The material, 10 g, was prepared at −18°.
*Crystalline 6,6′-di-*O*-tosylsucrose.
†On paper impregnated with silicic acid using the solvent system diisobutyl ketone:acetic acid:water (40:25:5).

TABLE III

Chromatography of "sucrose ditosylate"

	Tetra-*O*-tosylsucrose	Tri-*O*-tosylsucrose		Di-*O*-tosylsucrose	
		Fraction A	Fraction B	Fraction C*	Fraction D
Weight of material (g)	1.04	2.79	1.74	1.85	2.60
Per cent of mixture (w/w)	1.04	27.9	17.4	18.5	26.0
Sulphur content (%)					
Found:	12.96	11.82	11.76	10.12	10.11
Calc.:	13.38	11.93	11.93	9.85	9.85

NOTE: The material, 10 g, was prepared at 0°.
*Crystalline 6,6′-di-*O*-tosylsucrose.

The product formed on the ditosylation of sucrose was analyzed chromatographically. The results are presented in Table III and Fig. 2. A comparison of the yields reported in Figs. 1 and 2 shows that the ditosylation produced, as would be expected, a greater yield of 6,6′-di-*O*-tosylsucrose (fraction C). The yield of other sucrose ditosylates (fraction D) was, however, about the same as that obtained in the tritosylation. This result clearly indicates that the 6- and 6′-positions both undergo tosylation somewhat more rapidly than does the 1′-position. The fact that the ditosylation produced nearly as much 1′,6,6′-tri-*O*-tosylsucrose as did the tritosylation clearly reflects the greater reactivity of the three primary positions over the secondary positions of sucrose.

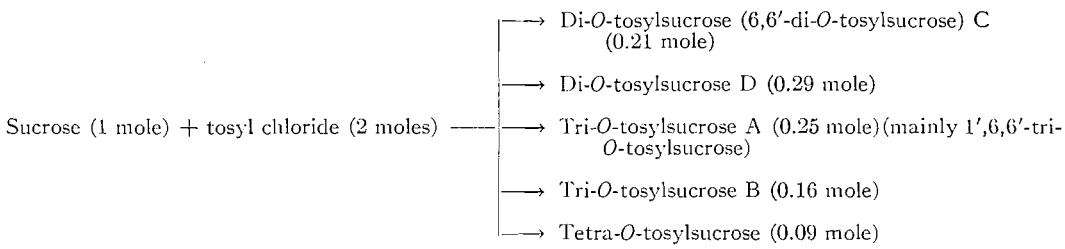

FIG. 2. Distribution of the tosylated sucroses formed in the reaction of 1 mole of sucrose with 2 moles of tosyl chloride in pyridine at 0°.

EXPERIMENTAL

The melting points are uncorrected and were determined on a microheating stage. Mallinckrodt reagent grade (100-mesh, lot 2847) silicic acid was used throughout.

"Tri-O-tosylsucrose"

A preparation was made at 0° in 95% yield using the method described by Hockett and Zief (9) and had a softening point of 65° to 85°, $[\alpha]_D$ +43° (*c*, 2.88 in chloroform). Anal. Calc. for $C_{33}H_{40}O_{17}S_3$: S, 11.93%. Found: S, 11.11%. The composition of this product is described in Table I and in Fig. 1.

A second preparation was made at −18° in a refrigerated bath using the following procedure. Sucrose, 34.2 g (0.1 mole), was dissolved in dry pyridine, 600 ml, by refluxing the mixture for a short time. The solution was then cooled to −18° with vigorous stirring for 2 hours. Tosyl chloride, 57 g (0.3 mole), was dissolved in 150 ml of dry pyridine and the solution was added dropwise to the solution of sucrose over a period of 2 hours. The reaction was left with continuous stirring at −18° for 5 days. The product, isolated in the usual manner, weighed 74.3 g (92.5%) with m.p. 65–85°, $[\alpha]_D$ +42° (*c*, 2.5 in chloroform). Anal. Calc. for $C_{33}H_{40}O_{17}S_3$: S, 11.93%. Found: S, 11.66%. The composition of this product is described in Table II.

Chromatography of "Tri-O-tosylsucrose" on Paper Impregnated with Silicic Acid

The silicic-acid-impregnated paper was prepared by the method of Marinetti, Erbland, and Kochen (16) but using Whatman No. 3 chromatographic paper. The chromatograms were developed by the descending method using the solvent system, diisobutyl ketone: acetic acid:water (40:25:5). The dried paper was immersed in 0.01% aqueous Rhodamine 6G solution (16) and examined under ultraviolet light in a dark room. Both "tri-*O*-tosylsucrose" preparations gave spots of similar intensities having R_f values of 0.15, 0.40, 0.65, and 0.90.

Silicic Acid-Column Chromatography of "Tri-O-tosylsucrose"

The silicic acid, 200 g, was packed as a slurry in chloroform in a 2.5-cm (diameter) column. The "tri-*O*-tosylsucrose", 9–10 g, was dissolved in 20 ml of chloroform and applied to the top of the column. The column was then developed with the solvent system, diisobutyl ketone:acetic acid:water (40:25:5). After the solvent front had displaced all of the chloroform from the column, 3-ml fractions were collected at a rate of 1 tube every 2 minutes. A total of 110 tubes were collected. Further eluant was free of material. The tubes were examined by spotting on paper and applying the Rhodamine 6G color test. Five different zones were apparent in the series of tubes. After the tubes within each zone had been combined, the solvent was removed *in vacuo* and the weights of the residues were determined.

The results are given in Tables I and II. Crystals were deposited in the fraction which proved to correspond to the fraction of di-*O*-tosylsucroses. In the case of the "tri-*O*-tosylsucrose" prepared at 0°, the crystalline substance (fraction C) was collected before evaporation of the solvent. The extremely fine needles, 0.92 g, m.p. 101–106°, were extremely soluble in methanol, but sparingly soluble in water, ethanol, or chloroform. After three recrystallizations from water, this compound melted at 108–110°, $[\alpha]_D$ +54° (*c*, 1.66 in ethanol). The compound only reduced Fehling's solution after acid hydrolysis. Anal. Calc. for $C_{26}H_{34}O_{15}S_2$: C, 47.8%; H, 5.22%; S, 9.85%. Found: C, 47.2%; H, 5.29%; S, 10.12%. The experiments described below established the compound to be 6,6′-di-*O*-tosylsucrose.

6,6′-Diiodo-6,6′-dideoxysucrose Hexaacetate

6,6′-Di-*O*-tosylsucrose (fraction C), 0.1 g, was dissolved in 1 ml of dry pyridine, and 2 ml of acetic anhydride and left at 4° for 2 days. The acetate, 126 mg, was isolated in the usual manner. The amorphous solid resisted crystallization. The substance, 76.5 mg, was dissolved in 15 ml of 10% sodium iodide in acetone and the solution was heated at 120° for 16 hours. The sodium tosylate, 36.2 mg, which precipitated represented a replacement of two tosyloxy groups. The syrupy product, 59.2 mg, was isolated in the usual manner. Its nuclear magnetic resonance spectrum was devoid of signals for the aromatic and *C*-methyl hydrogen atoms of the tosyl group. Anal. Calc. for $C_{24}H_{32}O_{15}I_2$: I, 31.4%. Found: I, 29.3%.

Monoiodomonodeoxymono-O-tosylsucrose Hexaacetate

The syrupy di-*O*-tosylsucrose (fraction D), 2.11 g, remaining after the removal of the crystalline 6,6′-*O*-tosylsucrose (see above) was acetylated and the product was subjected to the sodium iodide in acetone treatment. The amount of sodium tosylate recovered represented a replacement of 1.1 tosyloxy groups.

Reaction of Tri-O-tosyl Sucroses A and B with Sodium Iodide

Acetylated samples of tri-*O*-tosylsucrose fractions A, 135 mg, and B, 128 mg, were treated as described above with 10% sodium iodide in acetone. The amounts of sodium tosylate recovered were 49.69 mg and 41.58 mg, respectively. These amounts represent the replacement of 1.87 and 1.63 tosyloxy groups per mole, respectively. Anal. Calc. for $C_{29}H_{36}O_{16}I_2S$: I, 30.7%. Found: I, 28.6% (fraction A) and I, 27.8% (fraction B).

1′,2:3,6:3′,6′-Trianhydrosucrose Diacetate

Tri-*O*-tosylsucrose A, 200 mg, was dissolved in methanol *M* in sodium methoxide and the solution was refluxed for 30 minutes. The reaction mixture was evaporated to dryness and the residue was shaken with boiling pyridine, 50 ml, for 15 minutes. After the mixture was cooled to 0°, 10 ml of acetic anhydride was added. The syrupy product, isolated in the usual manner, crystallized on trituration with methanol. The yield was 71.6 mg (77.4% of theory) of 1′,2:3,6:3′,6′-trianhydrosucrose diacetate, m.p. 179–181°, undepressed when admixed with an authentic specimen.

Periodate Oxidation of the Tri-O-tosylsucroses A and B

To a sample of the tosyl ester, 0.05 millimole, dissolved in 80 ml of glacial acetic acid, 10 ml of 0.25 *N* sodium metaperiodate was added and the solution made up to 100 ml with water. Aliquots, 10 ml, were taken at suitable time intervals and were reacted with potassium iodide and an excess of 0.1 *N* sodium thiosulphate solution (18). The excess thiosulphate was determined by titration to the starch end point with standard

0.005 *N* iodine solution. Periodate was not consumed in the blank determinations. The periodate uptake by fraction A was 0.61, 1.53, 2.02, 2.18, 2.41, 2.78, and 2.82 moles of oxidant per mole of A after 0.5, 1, 10, 18, 32, 85, and 108 hours. The periodate uptake by fraction B was 0.49, 0.51, 0.98, 1.18, 1.59, 1.72, and 1.75 moles of oxidant per mole of B after 0.5, 1, 10, 18, 32, 85, and 108 hours.

Ditosylation of Sucrose

Sucrose, 34.2 g (0.1 mole), was dissolved in 600 ml of dry pyridine and cooled to 0°; tosyl chloride, 38 g (0.2 mole), was added and the reaction mixture was kept at 0° for 5 days. The reaction mixture was then shaken at room temperature with 5 ml water for 30 minutes. The excess pyridine was removed *in vacuo* at 40° to leave a thick syrup. This syrup was dissolved in 1 liter of chloroform and washed with 200 ml of ice-cold 2 *N* sulphuric acid followed by 200 ml of saturated sodium bicarbonate and finally with 200 ml of water. The chloroform solution, after drying over anhydrous sodium sulphate, was evaporated to a dry, amorphous solid, 56 g (86% of theory). Chromatography on silicic-acid-impregnated paper revealed three components, R_f values, 0.13, 0.38, and 0.6. Column chromatography of 10 g of the material allowed the isolation of the fractions described in Table III and in Fig. 2.

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REFERENCES

1. J. COMPTON. J. Am. Chem. Soc. **60**, 395 (1938).
2. R. C. HOCKETT and M. L. DOWNING. J. Am. Chem. Soc. **64**, 2463 (1942).
3. J. M. SUGIHARA. *In* Advances in carbohydrate chemistry. Vol. 8. Academic Press, Inc., New York.
4. R. S. TIPSON. *In* Advances in carbohydrate chemistry. Vol. 8. Academic Press, Inc., New York.
5. R. U. LEMIEUX and A. G. MCINNES. Can. J. Chem. **38**, 136 (1960).
6. I. BELLUCI. Gazz. chim. ital. **42**, II, 283 (1912).
7. T. P. HILDITCH and J. G. RIGG. J. Chem. Soc. 1774 (1935).
8. J. P. GIBBONS and R. A. JANKE. J. Am. Oil Chemists' Soc. **29**, 467 (1952).
9. R. C. HOCKETT and M. ZIEF. J. Am. Chem. Soc. **72**, 1839 (1950).
10. F. FINKELSTEIN. Ber. **43**, 1528 (1910).
11. A. L. RAYMOND and E. F. SCHROEDER. U.S. Patent 2, 365, 776 (1944).
12. P. D. BRAGG and J. K. N. JONES. Can. J. Chem. **37**, 575 (1959).
13. R. U. LEMIEUX and J. P. BARRETTE. J. Am. Chem. Soc. **80**, 2243 (1958).
14. R. U. LEMIEUX and J. P. BARRETTE. Can. J. Chem. **37**, 1964 (1959).
15. R. W. JEANLOZ. Abstracts of Papers, Am. Chem. Soc. **128**, 2D (1953).
16. G. V. MARINETTI, J. ERBLAND, and J. KOCHEN. Federation Proc. **16** (3), 837 (1954).
17. G. G. MCKEOWN, R. S. E. SERENIUS, and L. D. HAYWARD. Can. J. Chem. **35**, 28 (1957).
18. J. B. MARTIN. J. Am. Chem. Soc. **75**, 5483 (1953).