[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, HARVARD MEDICAL SCHOOL, AND THE MEDICAL SERVICES OF THE MASSACHUSETTS GENERAL HOSPITAL]

6-*O*-Methyl-D-galactosamine Hydrochloride (2-Amino-2-deoxy-6-*O*-methyl-D-galactose Hydrochloride)[1]

BY PIERRE J. STOFFYN AND ROGER W. JEANLOZ

RECEIVED APRIL 5, 1958

The synthesis of 6-*O*-methyl-D-galactosamine hydrochloride (2-amino-2-deoxy-6-*O*-methyl-D-galactose hydrochloride), a reference compound for structural studies of galactosamine-containing substances, is described. It was obtained in crystalline form *via* two independent routes and transformed into the crystalline *N*-acetyl, *N*-acetyl-tri-*O*-acetyl and *N*-(2′-hydroxynaphthylidene) derivatives.

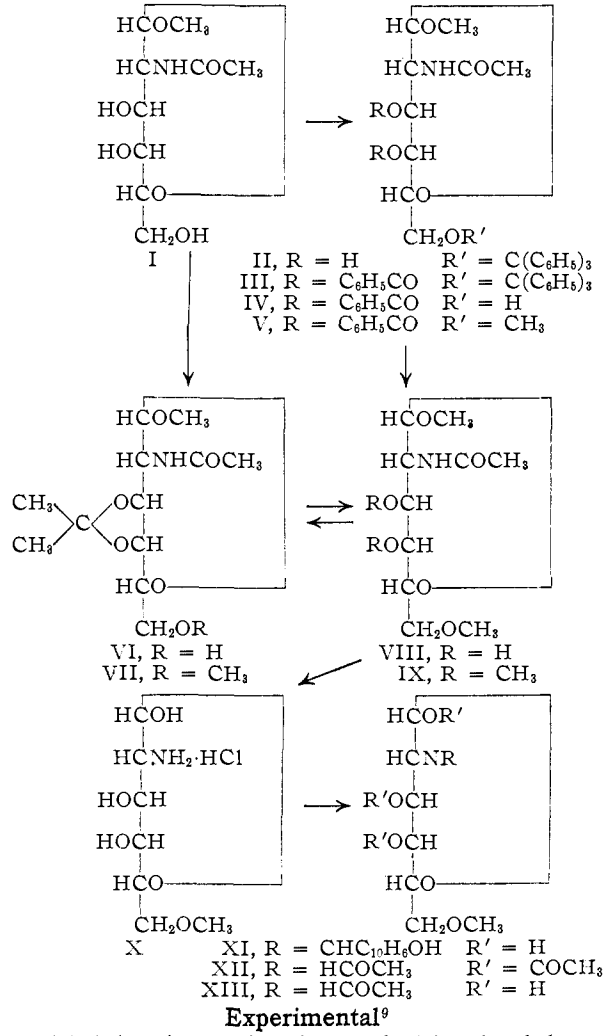
The isolation from the degradation products of methylated β-heparin[2] of a monomethyl derivative of 2-amino-2-deoxy-D-galactopyranose, different from the known 3-*O*-methyl[3] and 4-*O*-methyl[4] ethers, made of interest the synthesis of the remaining unknown 6-*O*-methyl derivative. The preparation of this compound was accomplished using two independent routes, as shown in the accompanying diagram.

In the first preparation, analogous intermediates were used as in the synthesis of 6-*O*-methyl-D-glucosamine[5]: namely, starting from methyl 2-acetamido-2-deoxy-α-D-galactopyranoside (I)[6] the 6-*O*-triphenylmethyl derivative II was prepared and subsequently was benzoylated in positions 3 and 4 (III). The triphenylmethyl group was hydrolyzed to give IV, the liberated hydroxyl in position 6 was methylated (V) and the protective benzoyl groups were removed to afford the crystalline methyl 2-acetamido-2-deoxy-6-*O*-methyl-α-D-galactopyranoside (VIII).

The same product VIII was obtained through a much shorter second route, by blocking positions 3 and 4 of I with an isopropylidene residue (VI), methylating the remaining free hydroxyl in position 6 (VII) and removing the protective isopropylidene group.

Transformation into the known 3,4,6-tri-*O*-methyl derivative IX,[6] as well as condensation with acetone to give the previously obtained isopropylidene derivative VII showed that no shifting of the ring had occurred during the formation of VIII. Further hydrolysis with hydrochloric acid gave the crystalline hydrochloride of 6-*O*-methyl-D-galactosamine (X), the α-form being assumed on the basis of the mutarotation.

This compound was further characterized by its transformation into a crystalline Schiff base by condensation with 2-hydroxynaphthaldehyde (XI)[7,8] and into a crystalline 1,3,4-tri-*O*-acetyl derivative (XII). Attempts to prepare the *N*-acetyl derivative XIII starting from the hydrochloride X gave only a trace of crystals. From the tetraacetate XII a crystalline product was obtained in small yield. Elementary analysis of this product corresponds to a monohydrate of XIII.



Experimental[9]

Methyl 2-Acetamido-2-deoxy-6-*O*-triphenylmethyl-α-D-galactopyranoside (II).—A mixture of 885 mg. of methyl 2-

(1) Aminosugars. XVIII (this heading replaces the one entitled "Studies on Hyaluronic Acid and Related Substances" previously used). This is publication No. 234 of the Robert W. Lovett Memorial for the Study of Crippling Disease, Department of Medicine, Harvard Medical School; address: Massachusetts General Hospital, Fruit St., Boston 14. This investigation has been supported by research grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service (Grant A-148-C3 and C4), and from the American Heart Association. It was presented before the Division of Carbohydrate Chemistry at the 132nd Meeting of the American Chemical Society, New York, N. Y., September, 1957.

(2) R. W. Jeanloz, P. J. Stoffyn and M. Trémège, *Fed. Proc.*, **16**, 201 (1957).

(3) P. J. Stoffyn and R. W. Jeanloz, THIS JOURNAL, **76**, 561 (1954).

(4) R. W. Jeanloz and P. J. Stoffyn, *ibid.*, **76**, 5682 (1954).

(5) R. W. Jeanloz, *ibid.*, **76**, 558 (1954).

(6) M. Stacey, *J. Chem. Soc.*, 272 (1944).

(7) Z. E. Jolles and W. T. J. Morgan, *Biochem. J.*, **34**, 1183 (1940).

(8) R. W. Jeanloz, THIS JOURNAL, **74**, 4597 (1952).

(9) R. W. Jeanloz, *ibid.*, **76**, 555 (1954); R. W. Jeanloz and D. A. Jeanloz, *ibid.*, **79**, 2579 (1957). Microanalysis by Dr. K. Ritter, Basel, Switzerland.

acetamido-2-deoxy-α-D-galactopyranoside (I) and 1.44 g. of triphenylchloromethane in 9 ml. of pyridine was kept for 36 hours at room temperature. The resulting homogeneous solution was heated for one hour at 100° and after cooling was poured on ice. The gummy precipitate was dissolved in chloroform and the solution was washed three times with ice-cold 10% potassium bisulfate solution, three times with water, dried over sodium sulfate and evaporated *in vacuo*. The residual sirup crystallized by addition of absolute ethanol. Recrystallization from the same solvent afforded 1.56 g. (88%) of short prisms changing into needles on storage. The prisms melted at 125–127° and recrystallized on further heating into needles, m.p. 178–181°, $[\alpha]^{27}D$ + 37± 1° (in chloroform, *c* 1.52). *Anal.* Calcd. for $C_{28}H_{31}O_6N$: C, 70.42; H, 6.54. Found: C, 70.48; H, 6.48.

Methyl 2-Acetamido-3,4-di-O-benzoyl-2-deoxy-6-O-triphenylmethyl-α-D-galactopyranoside (III).—A solution of 440 mg. of dry II in 4.4 ml. of anhydrous pyridine was cooled at −15° and 0.36 ml. of benzoyl chloride was added. The mixture was kept for one hour at 0° and then for 2 hours at room temperature. After addition of about 0.5 g. of ice which dissolved a crystalline precipitate of pyridine hydrochloride, the homogeneous solution was kept for 15 minutes and poured on 30 g. of ice. The microcrystalline precipitate formed on stirring was collected on a filter, then washed with water and dried (615 mg.).

Recrystallization from a mixture of acetone and ether gave 555 mg. (87%) of small rectangular prisms, m.p. 117–120°. At 150–160° the substance recrystallized in long needles melting at 175–177°. On a few occasions the substance recrystallized again in a third form melting at 192–193°, $[\alpha]^{24}D$ + 108 ± 2° (in chloroform, *c* 1.03). *Anal.* Calcd. for $C_{42}H_{39}O_8N$: C, 73.56; H, 5.74. Found: C, 73.40; H, 5.75.

Methyl 2-Acetamido-3,4-di-O-benzoyl-2-deoxy-α-D-galactopyranoside (IV).[10]—A solution of 665 mg. of III in 12 ml. of glacial acetic acid was heated on the water-bath and 6 ml. of water was added slowly. After one hour heating, the solution was evaporated *in vacuo* and the last traces of acetic acid were removed by codistillation with toluene. The partially crystalline residue was chromatographed on silicic acid. A mixture of benzene and ether 9:1 eluted 250 mg. (99%) of triphenylcarbinol, whereas IV was eluted by mixtures of ether and ethyl acetate 1:1 and pure ethyl acetate. Recrystallization from a mixture of acetone and ether gave 385 mg. (89%) of small prisms melting at 96–100°. The product recrystallized on further heating and melted at about 190°, $[\alpha]^{23}D$ +213 ± 3° (in chloroform, *c* 0.97). *Anal.* Calcd. for $C_{23}H_{25}O_8N$: C, 62.29; H, 5.68. Found: C, 62.26; H, 5.82.

Fifty milligrams of IV was treated with 0.5 ml. of dry pyridine and 0.3 ml. of acetic anhydride overnight at room temperature. The solvents were evaporated *in vacuo* by codistillation with toluene. The sirupy residue crystallized upon addition of a few drops of ether. Recrystallization from a mixture of acetone and ether gave 43 mg. (79%) of clusters of needles of **methyl 2-acetamido-6-O-acetyl-3,4-di-O-benzoyl-2-deoxy-α-D-galactopyranoside**, m.p. at 67–69°. On further heating the substance recrystallized in long needles, m. p. 92–93°, $[\alpha]^{23}D$ +182 ± 2° (in chloroform, *c* 0.84). *Anal.* Calcd. for $C_{25}H_{27}O_9N$: C, 61.85; H, 5.60. Found: C, 61.84; H, 5.52.

Methyl 2-Acetamido-3,4-di-O-benzoyl-2-deoxy-6-O-methyl-α-D-galactopyranoside (V).—A suspension of 283 mg. of IV in a mixture of 8 ml. of methyl iodide and 1.0 ml. of acetone was vigorously stirred at 35° for 4 hours with 0.3 g. of silver oxide. After a new addition of 0.3 g. of silver oxide, stirring was continued for 16 hours. The silver residue was collected on a layer of Celite and washed with ether. Evaporation of the filtrate *in vacuo* gave 129 mg. of sirup. Further extraction of the silver residue with warm methanol gave 164 mg. of residue which crystallized when seeded with the starting material. After drying, this product was methylated as described above and gave a second sirupy ether extract weighing 92 mg. Further extraction of the silver residue with warm methanol gave 80 mg., not further investigated. The two ether extracts were combined and chromatographed on silicic acid. Elution with chloroform gave 210 mg. (72%) of a colorless glass, $[\alpha]^{23}D$ + 192 ± 2° (in chloroform, *c* 0.75). *Anal.* Calcd. for $C_{24}H_{27}O_8N$: C, 63.01; H, 5.95; OCH_3, 13.57. Found: C, 62.97; H, 5.84; OCH_3, 13.50.

Methyl 2-Acetamido-2-deoxy-3,4-O-isopropylidene-α-D-galactopyranoside (VI).—A suspension of 1.55 g. of I in 150 ml. of acetone containing 0.2 ml. of concentrated sulfuric acid was shaken for 4 hours at room temperature. The homogeneous solution was neutralized with dry potassium carbonate, filtered and evaporated *in vacuo*. The partially crystalline residue was recrystallized from a mixture of methanol and acetone and gave 667 mg. of small prisms, m. p. 196–198°. The mother liquors concentrated to dryness were treated again with 100 ml. of acetone containing 0.16 ml. of concentrated sulfuric acid for 2.5 hours. Evaporation of the neutralized solution gave a residue which was chromatographed on silicic acid. Mixtures of ethyl acetate and acetone 4:1 eluted crystalline fractions from which 434 mg. of prisms were obtained by crystallization from a mixture of methanol and acetone, m.p. 196–198°. After two recrystallizations the melting point was raised to 198–198.5°, $[\alpha]^{23}D$ +156 ± 2° (in methanol, *c* 1.00). *Anal.* Calcd. for $C_{12}H_{21}O_6N$: C, 52.35; H, 7.69. Found: C, 52.33; H, 7.70. Further elution of the column with mixtures of acetone and methanol 9:1 gave 322 mg. of starting material. The total yield after deduction of the recovered starting material was 77%.

Various attempts to condense acetone using anhydrous copper sulfate and phosphorus pentoxide as catalyst resulted in lower yields.

Acetylation of 210 mg. of VI with acetic anhydride and pyridine in the usual way gave the **6-O-acetyl** derivative. By crystallization from a mixture of acetone, ether and pentane, 190 mg. (79%) of clusters of long needles were obtained, m.p. 143–144°, $[\alpha]^{23}D$ +156 ± 2° (in chloroform, *c* 1.00). *Anal.* Calcd. for $C_{14}H_{23}O_7N$: C, 52.99; H, 7.31. Found: C, 53.06; H, 7.20.

Methyl 2-Acetamido-2-deoxy-3,4-O-isopropylidene-6-O-methyl-α-D-galactopyranoside (VII). (a) **From VI.**—Four hundred and thirty milligrams of VI was methylated by refluxing for 8 hours in a mixture of 10 ml. of acetone and 30 ml. of methyl iodide in the presence of 1.0 g. of silver oxide. After distillation of most of the methyl iodide, the silver residue was collected on a layer of Celite and washed with acetone. The filtrate evaporated *in vacuo* gave a crystalline residue. Recrystallization from a mixture of acetone, ether and pentane yielded 379 mg. (84%) of diamond shaped platelets, m.p. 159–160°, $[\alpha]^{23}D$ +153 ± 2° (in chloroform, *c* 1.70). *Anal.* Calcd. for $C_{13}H_{23}O_6N$: C, 53.96; H, 8.01; OCH_3, 21.45. Found: C, 53.83; H, 7.98; OCH_3, 21.47.

Methylation of VI with dimethyl sulfate and sodium hydroxide gave VII in a lower yield.

(b) **From VIII.**—To a solution of 34 mg. of VIII in 5 ml. of acetone, 0.01 ml. of concentrated sulfuric acid was added. The mixture was kept for 3 hours at room temperature, then neutralized with a stream of dry ammonia. After filtration through Celite, the solution was evaporated and gave a partially crystalline residue. It was purified by chromatography on silicic acid. Elution with mixtures of ether and ethyl acetate, 4:1, 2:1 and 1:1, afforded 20 mg. (50%) of crystalline fractions. The product was recrystallized from a mixture of acetone and ether, m.p. 158–159°, and showed no depression of the m.p. in admixture with the compound obtained from VI.

Methyl 2-Acetamido-2-deoxy-6-O-methyl-α-D-galactopyranoside (VIII). (a) **From V.**—To a solution of 246 mg. of V in 5 ml. of methanol was added 0.5 ml. of 1.6 *N* barium methoxide. After standing overnight at 0°, the solution was diluted with 2 volumes of water and the barium ion was precipitated with carbon dioxide. After filtration through Celite, the solution was evaporated *in vacuo* and gave 137 mg. of crystalline residue. Recrystallization from a mixture of methanol, acetone and ether afforded 98 mg. (73%) of long needles in clusters, m.p. 207–208°, $[\alpha]^{23}D$ +164 ± 2° (in methanol, *c* 1.17). *Anal.* Calcd. for $C_{10}H_{19}O_6N$: C, 48.18; H, 7.68; OCH_3, 24.90. Found: C, 48.05; H, 7.57; OCH_3, 24.76.

(b) **From VII.**—A solution of 134 mg. of VII in 4 ml. of glacial acetic acid was heated on a steam-bath and 2.5 ml. of water was added slowly. After one hour the solution was evaporated *in vacuo*. Traces of acetic acid were eliminated by vacuum distillation in the presence of absolute ethanol. The crystalline residue was recrystallized from a mixture of methanol, acetone and ether and gave 83 mg. (72%) of

(10) This compound was obtained for the first time by Dr. D. M. Schmid.

needles, m.p. 206–207°. Admixture with material obtained from V did not depress the melting point.

Acetylation of 53 mg. of VIII with 0.5 ml. of acetic anhydride and 1.0 ml. of pyridine was carried out in the usual way. The sirupy residue obtained after evaporation of the solvents by codistillation *in vacuo* with toluene crystallized in the presence of small amounts of ether and pentane on cooling with Dry Ice. Purification by chromatography on silicic acid gave 62 mg. (87%) of the **3,4-di-*O*-acetyl** derivative. By recrystallization from a mixture of ether and pentane, 49 mg. (69%) of long needles were obtained, m.p. 83.5–85°, $[\alpha]^{24}D$ +101 ± 2° (in chloroform, *c* 0.99). *Anal.* Calcd. for $C_{14}H_{23}O_8N$: C, 50.44; H, 6.95. Found: C, 50.45; H, 7.01.

One hundred and thirteen mg. of crude VIII, obtained from 130 mg. of VII by hydrolysis with 60% acetic acid, was refluxed overnight with 4 ml. of acetone and 10 ml. of methyl iodide in the presence of 0.5 g. of silver oxide. After a new addition of 0.5 g. of silver oxide, reflux was continued for 10 hours. The suspension was filtered through a layer of Celite and the silver residue washed with acetone. After evaporation, the combined filtrates gave 114 mg. of a crystalline residue, further chromatographed on silicic acid. Mixtures of ethyl acetate and acetone 1:1 eluted 60 mg. of crystalline fractions. Recrystallization from a mixture of acetone, ether and pentane gave methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α-D-galactopyranoside (IX), m.p. 190–191.5°, showing no depression of melting point in admixture with authentic material[6]; $[\alpha]^{23}D$ +143 ± 2° (in chloroform, *c* 0.94).

6-*O*-Methyl-α-D-galactosamine Hydrochloride (2-Amino-2-deoxy-6-*O*-methyl-α-D-galactose Hydrochloride) (X).—A solution of 163 mg. of VIII in 5 ml. of 3 *N* hydrochloric acid was heated for three hours in a sealed tube at 100°. After cooling, the solution was evaporated *in vacuo* in the presence of absolute ethanol and gave 156 mg. of a crystalline residue. It was dissolved in methanol, the solution was filtered through a double layer of Darco G-60 and Celite, and concentrated under a stream of nitrogen to a volume of 2 ml. By addition of 4 ml. of acetone, 140 mg. (93%) of clusters of small prisms were obtained. The compound decomposed at 190–195° and showed mutarotation from $[\alpha]^{23}D$ +107.5° (after 5 minutes) to $[\alpha]^{23}D$ +92 ± 2° after 16 and 60 hours (in water, *c* 1.04). *Anal.* Calcd. for $C_7H_{16}O_5HCl$: C, 36.61; H, 7.02; Cl, 15.44; OCH_3, 13.51. Found: C, 36.42; H, 6.89; Cl, 15.47; OCH_3, 13.69.

2-Deoxy-2-(2′-hydroxynaphthylideneamino)-6-*O*-methyl-α-D-galactose (XI).—To a solution of 42 mg. of X in 1 ml. of water was added a solution of 90 mg. of 2-hydroxynaphthaldehyde and 60 mg. of $CH_3COONa \cdot 3H_2O$ in 6 ml. of methanol. The mixture was treated as previously described.[8] The residue was suspended in benzene and chromatographed on silicic acid. Elution with mixtures of ethyl acetate and acetone 1:1 and with pure acetone gave 59 mg. of crystalline fractions. Recrystallization of the main fractions from a mixture of methanol and acetone afforded 28 mg. (45%) of small yellow clusters of needles, m.p. 189–191°, with slight decomposition. The compound showed mutarotation from $[\alpha]^{23}_{5461}$ + 280° (after 20 minutes) to $[\alpha]^{27}_{5461}$ + 258 ± 3° after 17 and 45 hours (in methanol, *c* 0.98). *Anal.* Calcd. for $C_{18}H_{21}O_6N$: C, 62.24; H, 6.09. Found: C, 62.39; H, 6.15.

2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-methyl-α-D-galactopyranose (XII).—Crude X, obtained by hydrolysis of 150 mg. of VII, was acetylated with 2 ml. of acetic anhydride and 3 ml. of pyridine for 20 hours at room temperature. The crystalline residue, obtained after removal of the solvents by codistillation with toluene and absolute ethanol, was chromatographed on silicic acid. Mixtures of ether and ethyl acetate, 2:1 and 1:1, eluted 171 mg. of crystalline fractions. Recrystallization from a mixture of ethanol, ether and pentane gave 103 mg. (55%) of needles, m.p. 219–220°, $[\alpha]^{23}D$ +101 ± 2° (in chloroform, *c* 1.03). *Anal.* Calcd. for $C_{15}H_{23}O_9N$: C, 49.86; H, 6.42. Found: C, 49.94; H, 6.44.

2-Acetamido-2-deoxy-6-*O*-methyl-D-galactose (XIII).—To a solution of 75 mg. of XII in 1.5 ml. of methanol was added 0.15 ml. of 1.6 *N* barium methoxide. After standing 15 hours at 0° and 2 hours at room temperature, the solution was diluted with 2 volumes of water and neutralized by a stream of carbon dioxide. After filtration, traces of barium ion were removed by passing through a column of Dowex 50, and the solution was evaporated to dryness *in vacuo*. The residue crystallized from a mixture of methanol and acetone to give 15 mg. (30%) of fine needles, m.p. 165–168°, $[\alpha]^{24}D$ + 92 ± 5° (in water, *c* 1.12), no mutarotation being observed. *Anal.* Calcd. for $C_9H_{17}O_6N \cdot H_2O$: C, 42.68; H, 7.56. Found: C, 42.68; H, 7.63.

Acknowledgments.—The authors are indebted to Hoffmann–La Roche, Basel, for a generous gift of D-galactosamine hydrochloride.

BOSTON, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, HARVARD MEDICAL SCHOOL, AND THE MEDICAL SERVICES OF THE MASSACHUSETTS GENERAL HOSPITAL]

The Solvolysis of Sulfonyl Esters of Methyl α-D-Glucopyranoside and Methyl α-D-Altropyranoside[1]

BY ROGER W. JEANLOZ AND DOROTHY A. JEANLOZ

RECEIVED APRIL 5, 1958

The solvolysis in methyl Cellosolve solution in the presence of sodium acetate of sulfonyl (methylsulfonyl and *p*-tolylsulfonyl) esters in position 2 and 3 vicinal to a carboxyl (acetyl, benzoyl, α-ethyl-*n*-butyryl) ester of methyl α-D-glucopyranoside and methyl α-D-altropyranoside has been studied. Positions 4 and 6 were blocked by a benzylidene group or benzoyl groups. Walden inversion at the carbon linked to the sulfonyl group with retention of configuration at the carbon bearing the carboxyl ester was not observed. Hydrolysis of the carboxylic ester group took place, followed eventually by solvolysis of the sulfonyl group with epoxide formation and subsequent opening of the epoxide ring. The solvolysis in the presence of sodium methoxide of the methylsulfonyl and benzoyl esters at positions 2 and 3 of the 4,6-*O*-benzylidene derivatives was investigated.

In a series of papers started in 1942, Winstein and associates[2] have shown the influence of neighboring groups on the solvolysis of sulfonyl esters. By varying the solvolytic medium, they were able to effect solvolysis with or without retention of the configuration at the carbon linked to the sulfonyl group. These results were applied to the carbohydrate field by Baker and associates[3] and led to

(1) This is publication No. 235 of the Robert W. Lovett Memorial Foundation for the Study of Crippling Disease, Department of Medicine, Harvard Medical School; address: Massachusetts General Hospital, Fruit St., Boston 14. This investigation has been supported by research grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service (Grant A-148-C2 and C3). This work was presented in part before the Division of Carbohydrate Chemistry at the 128th Meeting of the American Chemical Society, Minneapolis, Minn., September, 1955.

(2) See S. Winstein, *et al.*, THIS JOURNAL, **64**, 2796 (1942), to **74**, 5584 (1952).

(3) B. R. Baker and R. E. Schaub, *ibid.*, **75**, 3804 (1953); B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. William, *ibid.*, **76**, 4044 (1954).