183°. The product showed mutarotation, from $[\alpha]^{21}D$ −84° (after 7 minutes) to $[\alpha]^{22}D$ −39 ± 1° (after 17 hours, in methanol, *c* 1.33). *Anal.* Calcd. for $C_{13}H_{19}O_5N$: C, 57.98; H, 7.11. Found: C, 57.96; H, 7.16.

3-*O*-Acetyl-1,6-anhydro-2,4-di-*O*-methyl-β-D-galactopyranose (V).—A solution of 760 mg. of 1,6:2,3-dianhydro-4-*O*-methyl-β-D-talose (III) in 50 ml. of methanol containing 1 g. of sodium was refluxed for 24 hours in a moisture-free system. After cooling, the solution was diluted with water, neutralized with carbon dioxide and evaporated *in vacuo* to dryness. The residue was extracted with hot chloroform. After evaporation of the solvent, 960 mg. of sirup remained, which was acetylated with 4 ml. of dry pyridine and 2 ml. of acetic anhydride, following the procedure described above for II. The residue was dissolved in benzene and chromatographed on silicic acid. Elution was obtained with mixtures of benzene and ether, 4:1 and 2:1. The first product was a sirup which was chromatographed a second time (see next paragraph). Crystalline fractions were obtained on elution with the 2:1 mixture. Recrystallization from a mixture of ether and pentane gave 765 mg. of prismatic needles of the D-galactose derivative V, m.p. 108°, $[\alpha]^{24}D$ −68 ± 2° (in chloroform, *c* 1.62). *Anal.* Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95; OCH_3, 26.72. Found: C, 51.64; H, 6.98; OCH_3, 26.68.

Chromatography of the non-crystalline fraction described above and of the mother liquors of V gave 15 mg. of V, and 115 mg. (10%) of a sirup (presumed to be mainly 1,6-anhydro-2-*O*-acetyl-3,4-di-*O*-methyl-β-D-idopyranose) with $[\alpha]^{27}D$ −86 ± 2° (in chloroform, *c* 0.91). *Anal.* Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.95; OCH_3, 26.72. Found: C, 51.81; H, 6.94; OCH_3, 26.64. Hydrolysis of this sirup and reaction of the resulting sirup with aniline gave besides 5 mg. of the anilide VIII only sirupy fractions. The total yield of V from III is thus 70%.

1,6-Anhydro-2,4-di-*O*-methyl-β-D-galactopyranose (IV).—To a solution of 400 mg. of V in 5 ml. of methanol was added 5 ml. of a solution of 0.4 *N* barium methoxide. After heating to boiling for two minutes, the solution was cooled, neutralized with sulfuric acid and evaporated *in vacuo* to dryness. The residue was extracted with chloroform, the solvent was evaporated and the residue, redissolved in chloroform, was chromatographed on silicic acid. Mixtures of chloroform and ether eluted fractions which gave by evaporation 315 mg. (96%) of a colorless sirup IV, $[\alpha]^{27}D$ −46 ± 2° (in chloroform, *c* 1.51). *Anal.* Calcd. for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.55; H, 7.51.

2,4-Di-*O*-methyl-α-D-galactopyranose (IX).—A solution of 205 mg. of IV in 5 ml. of 2 *N* sulfuric acid was heated overnight on a steam-bath. After addition of an excess of barium carbonate, the solution was filtered through a double layer of Darco G-60 and Celite. Evaporation *in vacuo* to dryness gave 223 mg. of a sirup which crystallized in the cold after addition of a few drops of 95% ethanol. Recrystallization from a mixture of 95% ethanol, acetone and ether gave 206 mg. (92%) of prisms, m.p. 105–108° (dried over P_2O_5). The compound crystallized as a hydrate and showed mutarotation from $[\alpha]^{27}D$ +113° (after 10 minutes) to $[\alpha]^{27}D$ +85 ± 2° (after 22 hours in water, *c* 1.15 for the dry product), indicating the product was the α-anomer.[13] *Anal.* Calcd. for $C_8H_{16}O_6 \cdot H_2O$: C, 42.47; H, 8.02; OCH_3, 27.44; H_2O, 7.96. Found: C, 42.43; H, 8.10; OCH_3, 27.40; H_2O, 7.98.

2,4-Di-*O*-methyl-*N*-phenyl-D-glucosylamine (VIII).—A solution of 61 mg. of IX in 2 ml. of absolute ethanol was refluxed with 28 mg. of aniline for two hours. The solution was then concentrated *in vacuo* and ether added to complete crystallization. Recrystallization from a mixture of methanol and ether gave 73 mg. (88%) of very small prismatic needles, m.p. 219–220°, $[\alpha]^{26}D$ +30 ± 3° (in methanol, *c* 0.68).[14] *Anal.* Calcd. for $C_{14}H_{21}O_6N$: C, 59.35; H, 7.47. Found: C, 59.29; H, 7.60.

Acknowledgments.—The author is indebted to Miss Ann Foley and to Miss Shirley Phillips for technical assistance.

(13) Baldwin and Bell[7] reported m.p. 100–103° and $[\alpha]^{20}D$ +85.7° (in water at equilibrium). Smith[8] reported m.p. 103° and $[\alpha]^{18}D$ +122.7° (after 5 minutes) mutarotating to +87.5° (in water at equilibrium).

(14) Smith[8] reported m.p. 216°.

BOSTON, MASSACHUSETTS

[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS AND SURGEONS AND THE EDWARD DANIELS FAULKNER ARTHRITIS CLINIC OF THE PRESBYTERIAN HOSPITAL]

Structural Studies on Chondroitin Sulfuric Acid.[1,2] I. The Nature of Chondrosine

BY EUGENE A. DAVIDSON AND KARL MEYER

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Chondrosine, a disaccharide from chondroitin sulfate of cartilage, has been prepared by an improved method and obtained in crystalline form for the first time. The disaccharide was shown to be 3(?)-(β-D-glucopyranosyluronic acid)-2-deoxy-2-amino-D-galactopyranose by reduction of the crystalline methyl ester hydrochloride with sodium borohydride to a 3(?)-(β-D-glucopyranosyl)-2-deoxy-2-amino-D-galactitol, demonstrated by the isolation of glucose pentaacetate after N-acetylation and acid hydrolysis. The N-acetyl derivative was hydrolyzed by β-glucosidase thus indicating the β-configuration of the disaccharide.

Chondrosine was isolated by Hebting[3] as the crystalline ethyl ester hydrochloride after hydrolysis of chondroitin sulfate with oxalic acid, and shown to be a desulfated and deacetylated disaccharide. Levene[4] isolated a crystalline methyl ester hydrochloride to which he assigned the structure of a chondrosaminidoglucuronic acid. Recently, Wolfrom, Madison and Cron[5] confirmed the results obtained by Levene and assigned to the disaccharide the structure 4-(2-amino-2-deoxy-β(?)-D-galactopyranosyl)-D-glucuronic acid. In contrast to these findings, in 1951 Masamune and co-workers,[6] on the basis of a positive Elson–Morgan reaction and periodate oxidation of N-acetylchondrosine ethyl ester, assigned to the disaccharide the structure 3-(D-glucopyranosyluronic acid)-2-deoxy-2-amino-D-galactopyranose. In previous publications from the same laboratory, various other structures had been reported.[7–9]

On the basis of the similarity of chondroitin sul-

(1) This work was supported by grants from the National Institutes of Health, the Helen Hay Whitney Foundation and the New York Chapter of the Arthritis and Rheumatism Foundation.

(2) Taken in part from a thesis to be submitted by Eugene A. Davidson in partial fulfillment of the requirments for the Ph.D. degree, Faculty of Pure Science, Columbia University.

(3) J. Hebting, *Biochem. Z.*, **63**, 353 (1914).

(4) P. A. Levene, *J. Biol. Chem.*, **140**, 267 (1941).

(5) M. L. Wolfrom, R. K. Madison and M. J. Cron, THIS JOURNAL, **74**, 1491 (1952).

(6) H. Masamune, Z. Yosizawa and M. Maki, *Tohoku J. Exp. Med.*, **55**, 47 (1951).

(7) H. Masamune and S. Osaki, *ibid.*, **45**, 121 (1943).

(8) T. Kobayashi, *ibid.*, **49**, 84 (1947).

(9) T. Nagaoka, *ibid.*, **53**, 29 (1950).

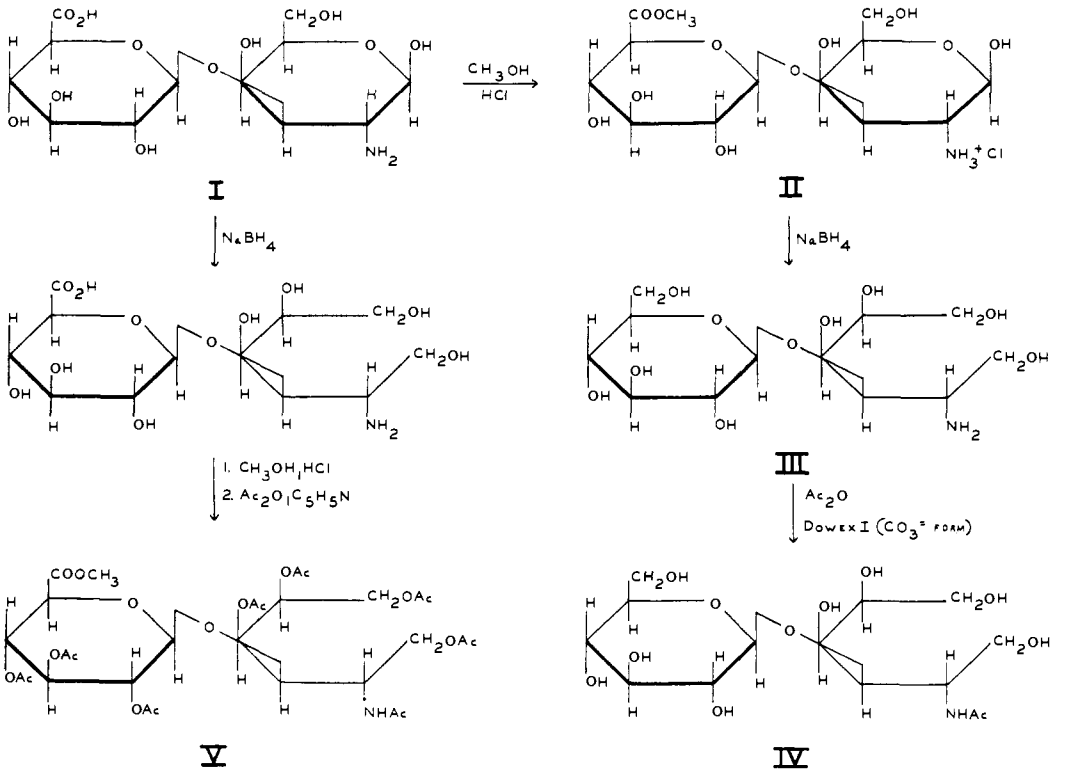

Fig. 1.

fate to hyaluronic acid as evidenced by the hydrolysis of both by testicular hyaluronidase and the work of this Laboratory in establishing the structure of the disaccharide obtained from hyaluronic acid by combined enzymatic and acid hydrolysis as a β-glucuronidoglucosamine,[10] a study of the disaccharide obtained from acid hydrolysis of chondroitin sulfate was undertaken.

Chondrosine, prepared according to Levene[4] by acid hydrolysis of the barium salt of chondroitin sulfate, showed four spots on paper chromatography, one corresponding to chondrosamine, one with the same R_f value as hyalobiuronic acid and two spots of less mobile material apparently representing higher molecular weight fractions. All four spots show the color reaction of Elson and Morgan,[11] typical of free 2-deoxy-2-amino sugars. With ninhydrin, all four spots show free α-amino groups. The chondrosamine in this mixture represents approximately one-third of the total.

Chondrosine was prepared by hydrolysis of the barium salt of chondroitin sulfate and fractionation of the hydrolysate on Dowex-50 (H^+ form)[12] (yield 61 to 67% based on uronic acid values). The amorphous material crystallized spontaneously from water as fine needles. The disaccharide was converted to the crystalline methyl ester hydrochloride with cold dilute methanolic hydrochloric acid.[13] The melting point and optical rotation of this material were similar to those described by Levene[4] and by Wolfrom, *et al.*[5] Chondrosine, on treatment with sodium borohydride, esterification and acetylation yielded methyl-(hepta-*O*-acetyl)-3(?)-(β-D-glucopyranosyluronate)-2-deoxy-2-acetamido-D-galactitol (V) identical in melting point and optical rotation with that described by Levene[4] and Wolfrom, *et al.*[5]

Chondrosine methyl ester hydrochloride was reduced with sodium borohydride and N-acetylated with acetic anhydride and Dowex-1 ($CO_3^=$ form) to give amorphous 3(?)-(β-D-glucopyranosyl)-2-deoxy-2-acetamido-D-galactitol. This material was hydrolyzed by β-glucosidase (emulsin)[14] thus establishing the β-configuration of the glucuronidic linkage. While only 15% of the theoretical reducing sugar was obtained after 48 hours, a slow rate of enzymatic hydrolysis of glucosides is not unusual.[15] α-Glucosidase did not hydrolyze the compound.

Compound IV was hydrolyzed and glucose isolated as the crystalline pentaacetate (yield 55%). The reaction scheme is summarized in Fig. 1 in which the linkage is provisionally assigned to the 3-position in analogy with hyalobiuronic acid.

The data reported above unequivocally establish that the disaccharide chondrosine is a β-D-glucuronidochondrosamine. The similarity in melting point and optical rotation of both the methyl ester hydrochloride and the acetylated reduced chondrosine methyl ester to those reported by Levene[4] and by Wolfrom and co-workers[5] leaves little doubt that all three laboratories were dealing with the same compound. No evidence could be found for the presence in the hydrolysis mixture of 2-deoxy-2-

(10) B. Weissman and K. Meyer, THIS JOURNAL, **76**, 1753 (1954).
(11) L. A. Elson and W. T. J. Morgan, *Biochem. J.*, **27**, 1824 (1933).
(12) Sulfonated Polystyrene Resin, Dow Chemical Co., Midland, Mich.
(13) E. F. Jansen and R. Jang, THIS JOURNAL, **68**, 1475 (1946).
(14) Salt-free emulsin, Mann Fine Chemicals, New York, N. Y.
(15) S. Veibel in Sumner and Myrback, "The Enzymes," Vol. I, Part I, Academic Press, Inc., New York, N. Y., 1950, p. 600.

amino-(D-galactopyranosyl)-D-glucuronic acid. As with hyaluronate, the hexosaminidic bond is preferentially hydrolyzed because the rate of deacetylation of the amino sugar is slower than the rate of glycosidic splitting.[16] Examination of the data of Wolfrom[5] reveals no inconsistency with the structure proposed in this paper.

Experimental[17]

Chondroitin Sulfuric Acid Salts.—Calcium chondroitin sulfate was prepared from tracheal cartilage powder by calcium chloride extraction as described by Meyer and Smyth.[18] Analysis: (Ca salt) uronic acid (CO_2) 30.7%; hexosamine 27.0%; sulfate 14.8%; N 2.61%, $[\alpha]^{24}D$ −28° (2, H_2O). Conversion to the barium salt was effected by dissolving the calcium salt in water and passage of the solution through a column of Dowex-50 (H^+ form). The effluent and aqueous washings were treated with the theoretical amount of barium acetate and lyophilized.

Preparation of Chondrosine (I).—Ten grams of calcium chondroitin sulfate (analysis above) was converted to the barium salt as described and hydrolyzed with 200 ml. of *N* sulfuric acid for four hours at 100°. A qualitative time study, followed by paper chromatography, indicated this to be the optimum hydrolysis time. Shorter periods of heating yielded increasing amounts of acetylated material while longer heating times increased the amount of chondrosamine formed. The solution was neutralized to *p*H 4.0 with saturated barium hydroxide; after removal of the barium sulfate, the pale yellow filtrate was placed on a column of Dowex-50 (H^+ form) (150 ml. settled volume), and the column washed with one liter of water. The chondrosine was eluted with 0.005 *N* acetic acid in a broad peak. The fractions containing ninhydrin positive material were concentrated to a small volume *in vacuo* and lyophilized. Paper chromatography of fractions so obtained indicated only one material to be present. Higher oligosaccharides and chondrosamine could be eluted from the column with 0.1 *N* hydrochloric acid; yield of chondrosine fraction, 3.80 g., 67% of theory based on the uronic acid of the starting material.

Anal. Calcd. for $C_{12}H_{21}O_{11}N$: N, 3.99; uronic acid, 55.0; hexosamine, 45.0, equiv. wt., 355.0. Found: N (micro-Kjeldahl), 3.98, amino N (ninhydrin) 3.92; uronic acid (carbazole), 54[19]; hexosamine, 42[20]; equiv. wt. (for mol), 352.0, $[\alpha]^{24}D$ +42° (amorphous, 2, water); the substance decomposed on heating. By paper chromatography in butanol–acetic acid–water (50:12:25 and 50:15:35) only one spot was observed with the same R_f as hyalobiuronic acid. The lyophilized material crystallized spontaneously from water as fine colorless needles. Recrystallized from aqueous ethanol, $[\alpha]^{24}D$ +40° (1, 0.05 *N* hydrochloric acid).

Preparation of Chondrosine Methyl Ester Hydrochloride (II).—An amount of 1.0 g. of chondrosine was treated with 36 ml. of cold 0.02 *N* methanolic HCl. The mixture was kept in the ice-box and shaken intermittently. After four days, the clear solution was evaporated to a small volume *in vacuo* and the hydrochloric acid removed by repeated additions of absolute ethanol and removal of solvents *in vacuo*. The chondrosine methyl ester hydrochloride crystallized during removal of solvent and was recrystallized twice from hot absolute ethanol; yield of recrystallized material 930 mg. (81% of theory), m.p. 159–161°, $[\alpha]^{23}D$ +42° (2, water).

Anal. Calcd. for $C_{13}H_{23}O_{11}NCl$: Cl, 8.74. Found: Cl, 8.71.

3(?)-(β-D-Glucopyranosyl)-2-deoxy-2-amino-D-galactitol (III).—Four hundred and eighty mg. of chondrosine methyl ester hydrochloride was dissolved in 10 ml. of absolute methanol, cooled to 5° and added in portions with occasional stirring to a cold solution of 137 mg. of sodium borohydride[21] in 10 ml. of 0.1 *M*, *p*H 8.0, borate buffer. The mixture was allowed to stand one hour at 5°, warmed to room temperature during the next two hours and acidified to *p*H 5.0 with acetic acid. Aliquots taken for analysis at this point showed: uronic acid (carbazole) 4.5%, glucose (anthrone)[22] 46%; reducing sugar,[23] Elson–Morgan reaction and Harding–McLean ninhydrin[24] were all negative. The Moore and Stein ninhydrin[25] reaction was positive. One ml. of glycerol was added, and, after standing in the ice-box overnight, the mixture was adsorbed on a column of Dowex-50 (H^+ form) (16 ml. settled volume). The column was washed first with 100 ml. of water, then by 100 ml. of 0.05 *N* acetic acid. The compound was eluted with 0.1 *N* H_2SO_4. The eluate was neutralized to *p*H 4.5 with saturated barium hydroxide, the barium sulfate removed by filtration and the filtrate and water washings lyophilized; yield 400 mg.

Anal. Reducing sugar (ferricyanide; hypoiodite[26]), less than 0.5%, uronic acid (carbazole), less than 1%; glucose (anthrone), 49%; Elson–Morgan or Harding–McLean reaction negative.

Hydrolysis of III with *N* sulfuric acid at 100° for 7 hours liberated only 10% of the theoretical reducing sugar. Paper chromatography of such a hydrolyzate in butanol–acetic acid–water, 50:12:25, and spraying with aniline trichloroacetate[27] indicated glucose as the only reducing sugar. β-Glucosidase (emulsin) had no hydrolytic action on III.

N-Acetyl Derivative of III (IV).—Three hundred mg. of III was stirred for 90 minutes with 5 ml. of water, 0.5 ml. of methanol, 6 ml. of Dowex-1 ($CO_3^=$ form) and 0.15 ml. of acetic anhydride.[28] At the end of this period the material was allowed to come to room temperature, the resin was removed by filtration and the filtrate and water washings passed through a small Dowex-50 (H^+ form) column. The combined aqueous eluates were lyophilized; yield 260 mg.

Anal. Glucose (anthrone), 45%; Moore and Stein ninhydrin and reducing sugar were negative.

β-Glucosidase (emulsin) hydrolyzed the above material in 0.1 *M*, *p*H 5.0, acetate buffer with the liberation of 15% of the theoretical amount of reducing sugar in 48 hours. Paper chromatography in butanol–acetic acid–water 50:12:25 and spraying with aniline trichloroacetate indicated glucose to be the only reducing sugar. Cellobiose, run as a control, was 60% hydrolyzed under identical conditions. α-Glucosidase (maltase)[29] liberated no reducing sugar from IV (ferricyanide method) nor was any reducing sugar demonstrable by paper chromatography. For comparison, maltose was subjected to the action of the α-glucosidase. Hydrolysis was complete in 24 hours as indicated by reducing sugar increase and paper chromatography.

Acid Hydrolysis of IV.—One hundred and eighty mg. of IV was hydrolyzed for four hours at 100° with 5 ml. of *N* sulfuric acid. After neutralization to *p*H 6.0 with saturated barium hydroxide and removal of barium sulfate followed by removal of the amino compound by passage through a 5-ml. column of Dowex-50 (H^+ form), the eluate and water washings were evaporated to dryness *in vacuo* and dried over phosphorus pentoxide *in vacuo*. The resulting glass was treated with 500 mg. of fused sodium acetate and 2 ml. of acetic anhydride for 90 minutes at 100°. Cracked ice was added and the mixture after shaking for one hour was neutralized to *p*H 7 with sodium carbonate and extracted with four 10-ml. portions of chloroform. Removal of the chloroform yielded a sirup which was crystallized from absolute methanol; yield 110 mg. 59% from IV, recrystallized twice from absolute methanol, m.p., 133.5–134°, no depression on admixture with authentic β-D-glucose pentaacetate, $[\alpha]^{26}D$ +2°, (1, $CHCl_3$).

Preparation of Methyl Hepta-*O*-acetyl-3(?)-(β-D-glucopyranosyl Uronate)-2-deoxy-2-acetamido-D-galactitol (V).—Three hundred and forty mg. of chondrosine was treated with 0.6 meq. of sodium carbonate in 15 ml. of water.

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(17) We wish to thank Dr. Alfred Linker for the gasometric uronic acid determination. Melting points are corrected.

(18) K. Meyer and E. M. Smyth, *J. Biol. Chem.*, **119**, 507 (1937).

(19) Z. Dische, *J. Biol. Chem.*, **167**, 189 (1947).

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(21) Metal Hydrides, Inc., Beverly, Mass.

(22) W. E. Trevelyan and J. S. Harrison, *Biochem. J.*, **50**, 298 (1952).

(23) O. Schales and S. S. Schales, *Arch. Biochem.*, **8**, 285 (1948).

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(25) S. Moore and W. H. Stein, *ibid.*, **176**, 367 (1948).

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(27) E. L. Hirst and J. K. Jones, *Discs. Faraday Soc.*, **7**, 268 (1949).

(28) S. Roseman and J. Ludowieg, THIS JOURNAL, **76**, 301 (1954).

(29) Bios Inc., New York, N. Y.

Eighty mg. of sodium borohydride in 10 ml. of water was added in portions with occasional stirring. The *p*H was maintained at 8.0 by the addition of *N* acetic acid when necessary. The mixture was allowed to stand for three hours, acidified to *p*H 5.0 with acetic acid and 360 mg. of sorbitol was added. The mixture, after standing in the ice-box overnight, was adsorbed on a 20-ml. column of Dowex-50 (H^+ form); the column was washed with 500 ml. of water and the disaccharide displaced with 5% pyridine. The uronic acid-containing portions were combined and lyophilized; yield 310 mg.

Anal. Reducing sugar—less than 1%; uronic acid (carbazole 52%); Elson–Morgan reaction negative.

Three hundred mg. of the reduced disaccharide was treated with 10 ml. of cold 0.02 *N* methanolic HCl and allowed to stand for 60 hours at ice-box temperature. The solvent was removed *in vacuo* and the HCl removed by repeated additions and evaporations of absolute ethanol. The resulting glass was treated with 7.5 ml. of pyridine and cooled to 0°. Five ml. of cold acetic anhydride was added and the mixture shaken for one hour at 0 to 5° and 1.5 hours at room temperature. The solvent was removed *in vacuo* and the resulting sirup was taken up in hot absolute ethanol from which crystals deposited on cooling; yield 121 mg., recrystallized from ethanol, m.p. 120.5–121° (cor.), $[\alpha]^{23}D$ −21° (1, $CHCl_3$). For this compound Levene[4] cites the constants m.p. 122°, $[\alpha]^{24}D$ −21° (3.2, ethanol) and Wolfrom, *et al.*,[5] report m.p. 121–123°, $[\alpha]^{21}D$ −23° (1.8, ethanol).

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Andromedotoxin. A Potent Hypotensive Agent from *Rhododendron maximum*

BY H. B. WOOD, JR., V. L. STROMBERG, J. C. KERESZTESY AND E. C. HORNING

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The isolation and characterization of andromedotoxin, a physiologically active agent present in leaves of *Rhododendron maximum*, is described. The nature of the oxygen-containing functional groups has been determined.

Descriptions of the toxicity of the leaves, leaf extracts, flower extracts and the honey from several species of the *Ericaceae* occur in old historical records, in agricultural bulletins, and in the chemical and pharmacological literature. The poisoning of Grecian soldiers by honey of *Rhododendron luteum* was recorded by Xenophon (*Anabasis*, Bk. IV), and the existence of a toxic agent in this plant was confirmed by Archangelsky[1] and Plugge.[2] The first systematic study was that of Eykman[3] who described under the name asebotoxin a toxic material from *Andromeda japonica*. An extensive review by Plugge[2] carried out at about the same time established the fact that comparable effects were shown by preparations isolated from leaves of several *Andromeda* species, and de Zaayer,[4] in Plugge's laboratory, later succeeded in isolating a physiologically active crystalline substance from leaves of *R. ponticum*. It was described as a neutral material, m.p. 228–229°, $[\alpha]^{12}D$ −14.2 (alc.), with a formula $C_{31}H_{51}O_{10}$. Following Plugge, it was given the name andromedotoxin. Archangelsky[1] confirmed the presence of a toxic agent in *R. ponticum* and *R. chrysanthum*, but the isolation and analysis of crystalline material was not reported again until 1921 when Hardikar[5] isolated a crystalline substance, m.p. 258°, from rhododendron leaves of unidentified species. The molecular formula was reported to be $C_{19}H_{30-32}O_6$, and the name andromedotoxin was retained by Hardikar. The optical rotation was not reported by Hardikar. Makino's investigation (of *R. hymenanthes*) gave still different results.[6] A neutral, crystalline, nitrogen-free product, m.p. 245°, was obtained. The formula of de Zaayer was supported, and the agent was renamed rhodotoxin. The most recent investigation, that of Gilfillan and Otsuki in 1938, dealt with *R. californicum*.[7] An unidentified non-toxic substance, m.p. 183.4°, was isolated,[8] but the physiological action was found to reside in a resinous fraction from which no well-defined material was isolated. There is uniform agreement in these papers that the plants in question contain an agent or agents toxic to many animal species, but the chemical data are limited in extent. The literature contains no substantial agreement on the physical properties or formulas of the materials which have been isolated, and no information about the structure of these compounds has been recorded. It is not known with certainty whether there are several agents of similar activity, or whether the same agent is present in each of the species which have been investigated.

The present study is concerned with a physiologically active substance present in leaves of *Rhododendron maximum*. It was isolated in the form of colorless needles whose melting point varied from 260 to 270° depending on the rate of heating. A change in crystal form occurred at 245–250°. The optical rotation was not widely different from that reported by de Zaayer, but the melting point behavior indicated a lack of identity with de Zaayer's andromedotoxin. The analytical data corresponded most closely to the formula of Hardikar, but the calculated carbon–hydrogen values for the de Zaayer–Makino substances fall near the same values.

Three crystalline derivatives of this substance were prepared, and the analytical data for these three derivatives and for the parent substance were

(1) K. Archangelsky, *Arch. exptl. Path. Pharmacol.*, **41**, 313 (1901).

(2) P. C. Plugge, *Arch. Pharm.*, **221**, 1, 813 (1883); **224**, 905 (1886).

(3) J. F. Eykman, *Rec. trav. chim.*, **1**, 225 (1882).

(4) H. G. de Zaayer and P. C. Plugge, *Arch. gesamte physiologie*, **40**, 480 (1886–1887).

(5) S. W. Hardikar, *J. Pharmacol. Exper. Therap.*, **20**, 17 (1922).

(6) M. Makino, *Okayama-Igakki-Zasshi*, **39**, 2099 (1927); **40**, 138 (1928); [*C. A.*, **23**, 1691, 3027 (1929)].

(7) F. A. Gilfillan and C. Otsuki, *J. Am. Pharm. Assoc.*, **27**, 396 (1938).

(8) The melting point of rhododendrin was reported by Archangelsky[1] to be 187°. It had no physiological activity.