mixture was extracted with cyclohexane and benzene. The water phase was evaporated to dryness *in vacuo*. The residue was kept at 40° for 3 hr. after addition of 1 *N* HCl (2 ml.). The PHT-DMAB precipitated was washed with a small amount of H_2O and dried.

The same method as above was employed to make PTH-3-methylproline. The PTH-3-methylproline was extracted with AcOEt after treatment with 1*N* HCl and dried.

Hydrochloric Acid Hydrolysis of Bottromycin A_2——Bottromycin A_2 (1 g.) was refluxed in 1*N* HCl (30 ml.) for 7 hr. The hydrolyzate was evaporated to dryness *in vacuo*, and the residue was dissolved in aqueous MeOH and passed through a column of IR 4B (OH type) to remove HCl. The eluate was dried *in vacuo* and purified by silica gel column chromatography. The Δ^1-isocaproyltetrapeptide was recovered from MeOH-AcOEt (2:8) eluate. Recrystallized from MeOH and ether, white prisms, m.p. 237~239°. Dried at 60° *in vacuo*. *Anal.* Calcd. for $C_{25}H_{43}O_5N_5$: C, 60.82; H, 8.78; O, 16.21; N, 14.19. Found: C, 60.85; H, 9.02; O, 16.06; N, 13.74. Dried at 150° *in vacuo*. *Anal.* Calcd. for $C_{25}H_{41}O_4N_5$: C, 63.13; H, 8.69; O, 13.46; N, 14.73. Found: C, 62.79; H, 9.10; O, 13.70; N, 14.50.

The dipeptide was eluted by MeOH-AcOEt=1:1 from the silica gel column and crystallized from MeOH, m.p. 190~194°. *Anal.* Calcd. for $C_{16}H_{19}O_3N_3S \cdot 1/2H_2O$. C, 56.11; H, 5.85; N, 12.28; S, 9.38. Found: C, 56.17; H, 5.90; N, 12.58; S, 9.21.

The dipeptide was hydrolyzed to 3-methyl-3-phenylalanine and 3-(2-thiazolyl)-β-alanine with constant boiling HCl at 120° for 17 hr.

Ozonolysis of the Δ^1-Isocaproyltetrapeptide——The Δ^1-isocaproyltetrapeptide (50 mg.) in $CHCl_3$ (20 ml.) and MeOH (2 ml.) was oxidized with O_3 at 0°. The same procedure before mentioned was traced and 2,4-dinitrophenylhydrazone of isobutylaldehyde was isolated.

Summary

Bottromycin A_2 is hydrolyzed to tetrapeptide and dipeptide by alkaline hydrolysis. The structures of both peptides are determined to be 3,3-dimethyl-2-aminobutylyl-valyl-3-methylprolylglycine and 3-methyl-3-phenylalanyl-3-(2-thiazolyl)-β-alanine.

Hydrochloric acid hydrolysis of bottromycin A_2 gives Δ^1-isocaproyltetrapeptide and the structure is discussed on the bases of pKa studies. The structure of bottromycin A_2 is proposed from the results above described.

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135. Toshio Kinoshita,*1,*2 Morizo Ishidate,*3 and Zenzo Tamura*1: 3-Ketoglucuronic Acid. I. Synthesis of 3-Ketoglucuronic Acid *4

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Recently, Imai and his co-workers[1] noted in their studies on nitric acid oxidations of starch that the oxidized product of starch contains some keto-sugar moieties. By periodate oxidation and subsequent acid hydrolysis of the product they[2] obtained

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*4 Preliminary communication of this work was published: M. Ishidate, Z. Tamura, T. Kinoshita: This Bulletin, **10**, 1258 (1962); **13**, 99 (1965).
1) Y. Imai: Yakugaku Zasshi, **81**, 1109 (1961); Y. Imai, Y. Hirasaka: *Ibid.*, **81**, 1362 (1961).
2) Y. Imai; *Ibid.*, **81**, 1115 (1961).

mesotartaric acid. This result provided convincing evidence for the presence of 3-keto-glucuronic acid moiety in the polysaccharide molecule. However, isolation of this ketouronic acid by means of acid hydrolysis was unsuccessful presumably because the anticipated keto-sugar should extremely be acid labile due to its 1,3-dicarbonyl structure.[3] 3-Ketoglucuronic acid would be a compound of particular interest because it is a novel type of carbohydrate having 1,3-dicarbonyl structure. In 1954, Lindberg and Theander[4] first isolated methyl 3-keto-D-glucopyranoside from the oxidation products of methyl D-glucopyranoside with dichromate. Theander and his collaborators[5] thereafter obtained several methyl 3-ketopyranosides. But as these keto-glycosides failed[6] to give corresponding free sugars by acid hydrolysis, chemistry of free 1,3-dicarbonyl carbohydrates has remained unknown.*[5] The present work was undertaken to synthesize free 3-keto-D-glucuronic acid starting from D-glucuronic acid. In consideration of the fact that 3-keto aldoses would be extremely unstable[7] against acid or alkali treatment and oxidation, the present investigation was first directed toward the synthesis of a suitably protected derivative of D-glucuronic acid, namely, methyl 1,2-O-isopropylidene-5-O-acetyl-D-glucofuranuronate (IId).

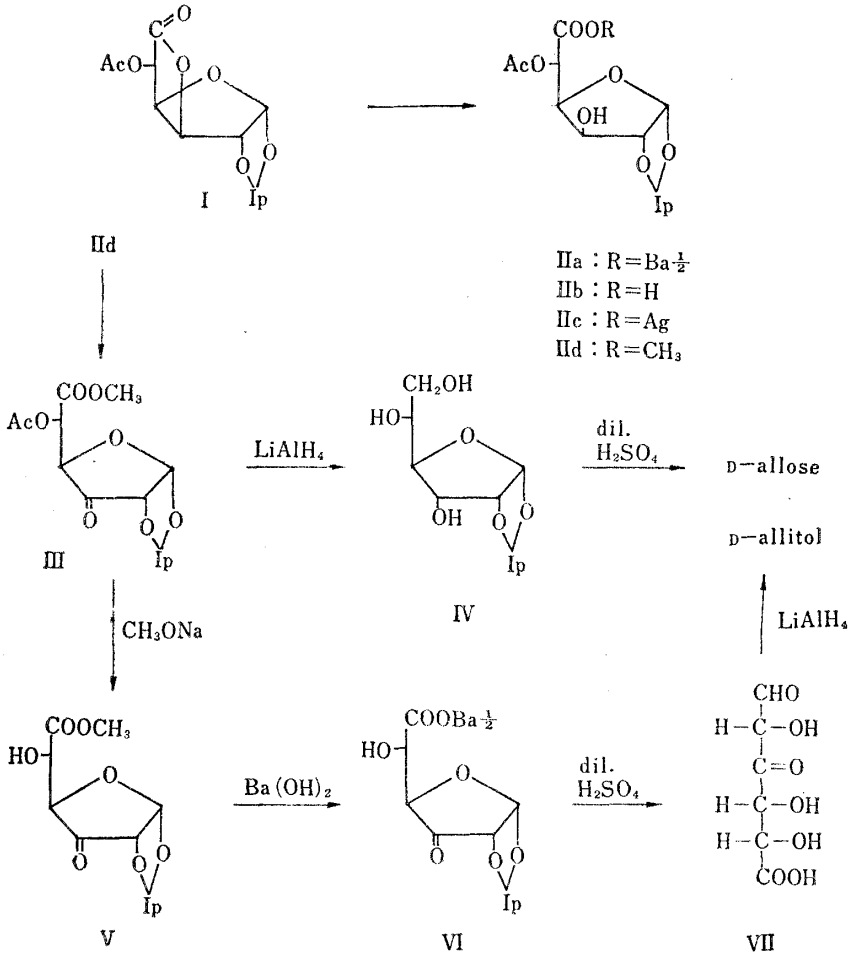

Chart 1.

*5 After the present work was initiated, it was reported that 3-keto-D-glucose was produced by fermentation. (cf. S. Fukui, R. M. Hochster : J. Am. Chem. Soc., **85**, 1697 (1963)) But the chemical properties or constitution of this sugar have little been informed.

3) Y. Hirasaka : Private communication.
4) B. Lindberg, O. Theander : Acta Chem. Scand., 8, 1870 (1954).
5) O. Theander : *Ibid.*, **11**, 1557 (1957); A. Assaraon, O. Theander : *Ibid.*, **12**, 1507 (1958).
6) O. Theander : *Ibid.*, **12**, 1897 (1958).
7) *Idem* : "Advances in Carbohydrate Chemistry," Academic Press, Inc. (New York), **17**, 279 (1962).

Treatment of 1,2-O-isopropylidene-5-O-acetyl-D-glucofuranurono-6,3-lactone(I)[8] with equivalent amount of barium hydroxide solution at low temperature gave barium 1,2-O-isopropylidene-5-O-acetyl-D-glucofuranuronate (IIa) which was characterized by its S-benzylthiuronium salt. Acetyl function at C-5 was little affected under the conditions employed. The structure of IIa was further confirmed on the fact that it was transformed with Amberlite IR-120 (H-form) into a free acid (IIb) which readily lactonized to regenerate I. IIa was then converted into methyl ester (IId) through the silver salt (IIc). Further experiment, however, revealed that IId was directly accessible from I by treatment with anion exchange resin in methanol in excellent yield.

Oxidation of IId with chromium trioxide in acetic acid afforded a crystalline product of empirical formula $C_{12}H_{16}O_8$. Infrared absorption spectrum of this substance indicated the presence of a newly introduced carbonyl group in the molecule. This oxidation product was assigned as methyl 3-keto-5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate (III) based upon the fact that it was reduced with lithium aluminum hydride to provide 1,2-O-isopropylidene-D-allofuranose (IV) as a sole product. The structure of IV was proved by its conversion into D-allose by acid hydrolysis. Interestingly the lithium aluminum hydride reduction of III took place stereo-selectively to give allose derivative (IV). This phenomenon is probably due to the steric hindrance caused by isopropylidene residue. This procedure may provide a convenient preparation for allose derivatives of furanoid type.

In acetic acid media, the yield of III in the above mentioned oxidation was poor because IId is easily convertible into the lactone (I). The oxidation in anhydrous acetone[5] or in pyridine gave no better result. Excellent results, however, were obtained when the oxidation was carried out in ethyl acetate under mild conditions. Ethyl acetate showed high advantage over the other usual solvents in its stability against the oxidant and in a favorable effect on the oxidation.

Removal of protective groups from III was accomplished by means of the following methods. Acetyl group of III was removed with sodium methoxide and the obtained methyl ester (V) was then hydrolyzed with barium hydroxide solution upon which barium 3-keto-1,2-O-isopropylidene-D-glucofuranuronate (VI) was isolated as a yellowish powder. Upon careful hydrolysis of VI with dilute sulfuric acid at low temperature, 3-keto-D-glucuronic acid (VII) was obtained. VII reduced ammoniacal silver nitrate solution and neutral potassium permanganate solution far more rapidly at room temperature than usual sugars. VII gave a single spot of Rf 0.32 on paper chromatogram using *n*-butanol-ethanol-water (2:1:1). Reduction of VII with lithium aluminum hydride gave D-allitol as a single product which was indistinguishable from authentic sample on electrophoretogram. D-Sorbitol, another possible isomer, was not detected in the reduction product. Crystalline 3-ketoglucuronic acid was found to contain dioxane as a crystallization solvent. Elemental analysis indicated that the molar ratio VII to dioxane was 1:1.

Experimental

All evaporations were carried out below 40° under reduced pressure unless otherwise stated.

Barium 5-O-Acetyl-1,2-O-isopropylidene-D-glucofuranuronate (IIa)——A solution of $Ba(OH)_2 \cdot 8H_2O$ (11.8 g.) in 200 ml. of water was added dropwise to a suspension of 5-O-acetyl-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone (I, 19.5 g.) in water (400 ml.) at 2~4° under mechanical stirring at such a rate as to maintain the pH of the reaction mixture below 9.0. After addition of alkaline solution was complete, the mixture was evaporated to dryness, the syrupy residue was extracted with $CHCl_3$ and the extract was evaporated to a yellowish powder of IIa (24.5 g., 96%) which showed $[\alpha]_D^{20}$ −6° (c=5.0, H_2O). S-benzylthiuronium salt of IIa was prepared in the usual way, m.p. 183~184°. (*Anal.* Calcd. for $C_{19}H_{26}O_8N_2S$: C,

8) Y. Nitta, J. Ide, A. Momose, M. Kawada: Yakugaku Zasshi, **82**, 790 (1960).

51.58; H, 5.92; N., 6.33. Found: C, 51.54; H, 5.71; N, 6.81).

5-O-Acetyl-1,2-O-isopropylidene-D-glucofuranuronic Acid (IIb)——A solution of IIa (1.0 g.) in minimum amount of water was passed through a column containing 20 ml. of Amberlite IR-120 (H-form) and lyophylized to afford IIb as a colorless syrup (810 mg., 75%). IIb gave positive hydroxamic acid-ferric chloride test indicating the presence of acetyl group and dissolved in 5% $NaHCO_3$ with evolution of CO_2. When the syrup (208 mg.) was heated on a boiling water bath at 4 mm. Hg for 20 min., I (120 mg.) was regenerated crystalline after recrystallization from EtOH.

Methyl 5-O-Acetyl-1,2-O-isopropylidene-D-glucofuranuronate (IId)——i) From IIa: A solution of silver sulfate (1.8 g.) in water (500 ml.) was gradually added to a solution of IIa (4.2 g.) in water (80 ml.) under stirring, precipitated barium sulfate was then removed by filtration and the filtrate was evaporated to a solid of silver 5-O-acety-1, 2-O-isopropylidene-D-glucofuranuronate (IIc). To this solid was added methyl iodide (10 ml.) dropwise under stirring over a period of 1 hr. and the mixture was stirred for further 2 hr. Precipitated silver iodide was filtered off and the resulting solution was evaporated to a syrup, which was triturated with ether and precipitates were recrystallized from toluene to give 1.5 g. (42% from I) of product which showed m.p. 105~106°, and $[\alpha]_D^{20}$ −14° (c=6.3, MeOH) (*Anal.* Calcd. for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25. Found: C, 49.85; H, 6.59.)

ii) From I: To a solution of I (50 g.) in MeOH (400 ml.) added Amberlite IR-45 (OH-form) and the mixture was stirred for 2 hr. at room temperature. The resin was then filtered off and the filtrate was concentrated to a syrup which was triturated with 15 ml. of dry toluene and deposited crystals were collected and washed with a minimum amount of toluene to give 25.8 g. (45%) of IId, m.p. 104~105°. This product was used for preparation of III without further purification.

Methyl 3-Keto-5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate (III)——i) By oxidation of IId with chromium trioxide in acetic acid: To a solution of CrO_3 (6.5 g.) in acetic acid (21 ml.) containing 4.5 ml. of water on an ice bath was added IId (4.5 g.) portionwise over a period of 1.5 hr. under stirring. The resulting mixture was stirred at room temperature for further 2 hr., then allowed to stand at 38° overnight and poured into ice water containing 2 g. of $NaHCO_3$. The mixture was extracted with $CHCl_3$ (30 ml. × 5). Combined extracts were washed successively with 5% $NaHCO_3$ and water, dried with Na_2SO_4 and evaporated to a syrup (1.4 g.). The syrup was triturated with ether and deposited crystals were collected. Recrystallization from isopropyl ether afforded 0.42 g. of III, m.p. 102~103°, $[\alpha]_D^{20}$ +132° (c=1, $CHCl_3$) IR cm^{-1} ($CHCl_3$): $\nu_{C=O}$ 1783 (carbonyl group at C-3) $\nu_{C=O}$ 1750, ν_{C-H} 1440 (methylester) (*Anal.* Calcd. for $C_{12}H_{16}O_8$: C, 50.00; H, 5.60. Found: C, 50.02; H, 5.86). The mother liquor from which IId was separated was submitted to thin-layer chromatography using toluene-acetone-$CHCl_3$ (3 : 1 : 2). After heating the plate at 150° for 15 min. three spots were observed. The spots corresponded to I (Rf 0.82, yellow), IId (Rf 0.48, yellow) and III (Rf 0.70, brown). On evaporation of the mother liquor, 0.33 g. of I was recovered crystalline.

ii) By oxidation of IId with chromium trioxide in ethyl acetate: To a solution of IId (50 g.) in 150 ml. of AcOEt was added finely powdered CrO_3 (50 g.) under stirring at such a rate as the temperature of the reaction mixture was maintained in the range of 38~40°. After addition of the oxidant, stirring was continued for further 2 hr. and the mixture was allowed to stand overnight at room temperature. The brown precipitate of chromium oxide was filtered off and washed with AcOEt. The filtrate and washings were passed successively through a column containing 75 g. of Al_2O_3 and evaporated to a syrup which spontaneously crystallized. On recrystallization from isoprophyl ether, 21.5 g. (43%) of III was obtained.

Reduction of Methyl 1,2-O-Isopropylidene-5-O-acetyl-3-keto-D-glucofuranuronate (III) with Lithium Aluminum Hydride——To a solution of III (400 mg.) in ether (20 ml.) placed on an ice bath was added a suspension of $LiAlH_4$ (400 mg.) in ether (30 ml.) under vigorous stirring and the stirring was continued for additional 30 min. AcOEt (20 ml.) and water (1 ml.) were added successively to the mixture at intervals of 30 min., the precipitates were filtered off and washed with water. The combined filtrate and washings were passed through a column containing Amberlite IR-120 (H-form, 10 ml.) and another column containing Amberlite IRA-411 (OH-form, 10 ml.) successively, and evaporated to a crystalline residue, which was washed with ether. Recrystallization from EtOH-ether gave 142 mg. (54%) of 1,2-O-isopropylidene-D-allofuranose (IV), m.p. 133° (*Anal.* Calcd. for $C_9H_{16}O_6$: C, 49.08; H, 7.32. Found: C, 48.43; H, 7.23). IV showed distinct depression of melting point when admixed with 1,2-O-isopropylidene-D-glucofuranose. Rf value of IV was 0.92 when paperchromatographed in *n*-BuOH-pyridine-H_2O (6 : 4 : 3) whereas 1,2-O-isopropylidene-D-glucofuranose showed Rf value of 0.86 and these two compounds were distinctly discriminated by mixed application technique. The mother liquor, from which IV was separated, was paperchromatographed in the same solvent and showed a single spot of IV.

Hydrolysis of IV——IV (40 mg.) was dissolved in 1% H_2SO_4 (1.2 ml.) and heated on a boiling water bath for 2 hr. The solution was neutralized with $BaCO_3$ and evaporated to a syrup which crystallized (26 mg.) on addition of acetone. This product showed no depression of melting point when admixed with authentic D-allose and indistinguishable with authentic D-allose by paper electrophoresis in basic lead acetate buffer according to Frahn and Mills.[9]

9) J. L. Frahn, J. A. Mills: Australian J. Chem., **1959**, 65.

3-Keto-D-glucuronic Acid (VII)——To a solution of III (10 g.) in 20 ml. of dry MeOH was added 0.2 *N* NaOMe (20 ml.) and the resulting mixture was stood on an ice bath for 3 hr. Sodium ion was then removed with Amberlite IR-120 (H-form, 6 g.) and the solution was evaporated to a pale yellow syrup which should be methyl 3-keto-1,2-O-isopropylidene-D-glucofuranuronate (V). The syrup was then dissolved in a small amount of water and to this solution was added 7 % barium hydroxide solution (78 ml.) at such a rate that the pH of the reaction mixture was maintained below 9.0 at room temperature. The mixture was decolorized with charcoal and evaporated to a yellow powder (VI, 9.7 g., 93 %) IR cm^{-1} (KBr): 1770 (C=O at C-3), 1600 (carboxylate at C-6), no carbonyl peaks of esters.

To a solution of VI (7.8 g.) in 50 ml. of water, added 3.5 ml. of 45 % sulfuric acid at 0° and the mixture was left at 5~10° for 5 days. The mixture was then stirred with 4.8 g. of $BaCO_3$ and as soon as the pH reached 3.0, filtered immediately. The filtrate was passed through a column containing Amberlite IR-120 (H-form, 10 ml.) and concentrated under the pressure of 4 mm. Hg on a water bath below 20° to a syrup. The evaporation was further continued with occasional addition of dry dioxane. Finally, the resulting thick syrup was dried over P_2O_5. Trituration of the syrup with a mixture of dry dioxane (0.8 ml.) and dry acetone (2.0 ml.) gave crystalline 3-keto-D-glucuronic acid (VII) which was collected after storing in a refrigerator for a week, 1.2 g., (15.4 % from III). On recrystallization from acetone-dioxane (2:1), VII showed m.p. 97~98°, and mutarotation $[\alpha]_D^{20}$ +50 to −40°. (*Anal.* Calcd. for $C_6H_8O_7 \cdot C_4H_8O_2$: C, 42.86; H, 5.74. Found: C, 42.50; H, 5.72.)

Reduction of VII with $LiAlH_4$——To a suspension of $LiAlH_4$ (100 mg.) in dioxane (30 ml.) placed on an ice bath was added VII (100 mg.) and the resulting mixture was stirred for 1 hr. at room temperature and the stirring was continued for additional 2 hr. at 50°. Acetone (10 ml.) and water (10 ml.) was added successively to the mixture at intervals of 30 min. with external cooling on an ice bath. The mixture was then filtered, the filtrate was passed through a column containing Amberlite IR-120 (H-form, 20 ml.) and another column containing Amberlite IRA-411 (OH-form, 10 ml.) successively and evaporated to a colorless syrup. The obtained syrupy material was dissolved in a minimum amount of water and submitted to electrophoresis in basic lead acetate buffer according to Frahn and Mills.[9] The product was indistinguishable with D-allitol and unequivocally discriminated from D-sorbitol and D-mannitol by mixed application technique.

Proof of Dioxane contained in the Crystals of VII——Several mg. of the crystals of VII were taken in a glass capillary tube placed horizontally on a hot plate. The middle of the tube was narrowed, the open end was inserted into a barium hydroxide solution. On heating the sealed end of the tube, vigorous bubbling occurred, colorless liquid distilled at the narrow part of the tube and distinct turbidity of barium hydroxide solution indicated evolution of CO_2. The liquid material was submitted to IR spectroscopy as a $CHCl_3$ solution and to gas chromatography using polyester succinate as a stationary phase that proved identical with authentic dioxane.

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Summary

3-Ketoglucuronic acid, a new 1,3-dicarbonyl carbohydrate, was synthesized. 5-O-Acetyl-1,2-O-isopropylidene-D-glucofuranurono-6,3-lactone (I) was converted into methyl 5-O-acety-1,2-O-isopropylidene-D-glucofuranuronate (IId), which was oxidized with chromium trioxide to give 3-keto-5-O-acetyl-1,2-O-isopropylidene-D-glucofuranuronate (III). Ethyl acetate was found to be an excellent solvent for this oxidation. Removal of protective groups from III afforded crystalline 3-ketoglucuronic acid (VII).

The intermediate substance (III) underwent stereo-selective reduction by the action of lithium aluminum hydride to produce 1,2-O-isopropylidene-D-allofuranose.

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