Biochemical Studies on "Bakanae" Fungus. Part 69.

Synthesis of Substances related to Gibberellins

Part XII* Reactions of Compounds derived from Gibberellin C

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Degradation of gibberellin C afforded methyl 1,7-dimethyl-2 α -hydroxy-8-oxo-7 α -gibb-4a(4b)-ene-1,10-dicarboxylate, from which gibberellin C was reconstructed. Reactions of desoxogibberellin C are also described.

It has been shown that gibb-4a(4b)-ene-1,10-dicarboxylates smoothly relactonize to afford gibbane 1 $\rightarrow$ 4a-lactonic acids¹⁾. This paper describes further examples of relactonization as well as several new compounds derived from gibberellin C.

2(eq)-Hydroxy-epimer of Gibberellin C²⁾ (Isogibberellin A₁³⁾).

It is well established that 2(ax)-hydroxy-gibbane 1 $\rightarrow$ 4a-lactones are epimerized in dilute aqueous alkali to the more stable 2(eq)-hydroxy-compounds^{2,3)}. Thus gibberellin C (Ia) gives the 2(eq)-hydroxy-epimer²⁾ (IIa, isogibberellin A₁³⁾) by treatment with dilute alkali. A gibb-4a(4b)-ene diester (IIIa), derived from the 2(eq)-hydroxy-epimer (IIa) is now converted to gibberellin C. This constitutes a part of the efforts directed toward the total synthesis of gibberellin C.

2(eq)-Hydroxy keto ester (IIb) afforded oily 2(eq)-hydroxy keto diester (IIIa) when

gibberellin A₁ methyl ester, the existence of The infrared spectrum of the corresponding 2(eq)-acetate (IIIb), m.p. 137~138°C, was clearly different from that of the known 2(ax)-acetate (Vb)⁴⁾. The assigned structure (IIIa) was supported by the fact that the oily 2(eq)-hydroxy ester, upon oxidation, gave a crystalline diketo ester which was identical with the known diketo ester (IV)⁵⁾ derived from 2(ax)-hydroxy diester (Va). Treatment of 2(eq)-hydroxy diester (IIIa) with aqueous sulfuric acid followed by ethereal diazomethane afforded the expected relactonized product, the 2(eq)-hydroxy keto ester (IIb).

The next problem was the conversion of the 2(eq)-hydroxy keto ester (IIb) to gibberellin C methyl ester (Ib). In case of gibberellin A₁ methyl ester, the existence of an equilibrium between the 2-hydroxy-epimers has already been shown by the British workers²⁾. The same situation was expected to exist in case of gibberellin C methyl ester, too. Actually about 10% of gibberellin C

* Part XI, K. Mori, M. Matsui and Y. Sumiki, This Journal, **28**, 72 (1964).

1) K. Mori, M. Matsui and Y. Sumiki, This Journal, **27**, 530 (1963).

2) B.E. Cross, J.F. Grove and A. Morisson, *J. Chem. Soc.*, **1961**, 2498.

3) N. Takahashi, Y. Seta, H. Kitamura, A. Kawarada and Y. Sumiki, This Journal, **23**, 493 (1959).

4) D.C. Aldridge, J.F. Grove, R.N. Speake, B.K. Tidd and W. Klyne, *J. Chem. Soc.*, **1963**, 143.

5) B.E. Cross, *ibid.*, **1960**, 3022.

methyl ester (Ib) was isolated when the 2(eq)-hydroxy-epimer (IIb) was shaken with 0.01 N-sodium hydroxide. Treatment of the former with dilute hydrochloric acid gave gibberellin C (Ia). This concluded the conversion of 2(eq)-hydroxy diester (IIIa) to gibberellin C (Ia).

Desoxogibberellin C. In connection with other synthetic studies, gibberellin C derivatives were prepared in which C-8 keto group was reduced to methylene.

Both gibberellin C methyl ester (Ib) and its acetate (Ic) gave the corresponding thio-

ketals (VIa, m.p. 222~223°C, and VIb, oil) when treated with ethanedithiol and boron trifluoride etherate. Desulfurization of these compounds with Raney nickel W-7 in dioxane afforded desoxogibberellin C methyl ester (VIIb), m.p. 159~160°C, and its acetate (VIIc), m.p. 134~135°C. The acetate (VIIc) was also obtained by acetylation of the hydroxy ester (VIIb). Chromic acid oxidation of the hydroxy ester (VIIb) gave a keto ester (VIII), m.p. 132~133°C. The hydroxy ester (VIIb) was hydrolyzed with difficulty by dilute hydrochloric acid to afford impure

desoxogibberellin C (VIIa), m.p. 205~210°C, in poor yield.

The oily thioketal (IX) of 2(ax)-hydroxy diester (Va) was desulfurized to give a desoxo diester (X), m.p. 136~137°C. This was shown to be identical with the methanolysis product of desoxogibberellin C methyl ester (VIIb). Relactonization of this diester (X) with hot aqueous sulfuric acid afforded a very small amount of the expected lactone as its methyl ester (VIIb). Desoxo compounds so far examined, including synthetic materials such as the ester (XI)⁶, were all acid-labile, suggesting their tendency to undergo skeletal rearrangements with acid.

EXPERIMENTAL

All melting points were not corrected. Infrared spectra were measured as Nujol mulls unless otherwise stated.

Methanolysis of the 2(eq)-Hydroxy Keto Ester (IIb). A solution of the ester (IIb, 600 mg) in dry methanol (30 ml) saturated with hydrogen chloride was heated under reflux for 4 hrs in a slow stream of hydrogen chloride. The solution was poured on ice and extracted with ethyl acetate. To the extract was added ethereal diazomethane. The solution was washed with water and dried over sodium sulfate. Recovery gave a gum which was chromatographed on alumina (15×2.5 cm) in benzene. Elution with benzene-ether (1:1, 500 ml) afforded a small amount of intractable gum (about 50 mg, Beilstein test positive). Ether (1 l) eluted nothing. Elution with benzene-methanol (150:1, 150 ml after 350 ml of forerun) gave the oily diester, methyl 1,7-dimethyl-2 α -hydroxy-8-oxo-7 α -gibb-4 α (4b)-ene-1,10-dicarboxylate (IIIa, about 300 mg). All attempts to crystallize this failed. The oil shows different infrared spectrum from that of the 2(ax)-hydroxy-epimer. $\nu_{\max}$ (liquid film) 3520, 1735, 1720 cm^{-1} . **Acetate (IIIB).** The oily diester (IIIa) was acetylated with acetic anhydride and pyridine. Elongated prisms from ether-petroleum ether, m.p. 137~138°C. *Anal.* Found: C, 66.05; H, 6.92. Calcd. for $\text{C}_{28}\text{H}_{40}\text{O}_7$: C, 66.01; H, 7.23%. $\nu_{\max}$ 1738, 1718 cm^{-1} . Further elution with benzene-methanol (150:1, 250 ml) gave a small amount of gum. Then elution with

benzene-methanol (150:1, 100 ml and 50:1, 300 ml) afforded the starting material (IIb, 100 mg).

Oxidation of the 2(eq)-Hydroxy Diester (IIIa) to the Diketo Diester (IV). To a solution of the hydroxy diester (IIIa, 30 mg) in acetone (1 ml) was added Jones' chromic acid reagent⁷ (0.05 ml) at 0°C and the mixture was left to stand at room temperature for 1.5 hrs. Then the mixture was diluted with water and extracted with ether. Removal of ether followed by trituration of the residue with methanol afforded needles of methyl 1,7-dimethyl-2,8-dioxo-7 α -gibb-4 α (4b)-ene-1,10-dicarboxylate (IV). Needles from ethyl acetate-petroleum ether, m.p. 116~118°C. $\nu_{\max}$ 1730, 1715, 1220, 1070 cm^{-1} . Mixed m.p. with the diketo diester prepared from 2(ax)-epimer (Va) showed no depression. The infrared spectrum was identical with that of the ester derived from 2(ax)-epimer (Va).

Relactonization of the 2(eq)-Hydroxy Diester (IIIa) to the 2(eq)-Hydroxy-Epimer (IIb) of Gibberellin C Methyl Ester. A mixture of the diester (IIIa, about 190 mg), methanol (2 ml), water (10 ml) and concd. sulfuric acid (3 ml) was refluxed for 4.5 hrs. After cooling, the mixture was extracted with ethyl acetate. The extract was washed with water, treated with ethereal diazomethane, dried and evaporated. The residue crystallized from ethyl acetate-petroleum ether to give needles of the desired lactone (IIb), m.p. 222~225°C. Yield, 80 mg. $\nu_{\max}$ 3560, 1756, 1730 cm^{-1} . This was identified with an authentic sample of the 2(eq)-hydroxy-epimer (IIb) by mixed m.p. and infrared measurement. The starting diester (IIIa) was recovered from the mother liquor.

Conversion of the 2(eq)-Hydroxy-Epimer (IIb) to Gibberellin C Methyl Ester (Ib). The 2(eq)-hydroxy keto ester (IIb, 250 mg) was shaken with 0.01 N-sodium hydroxide (150 ml) for 2 hrs at room temperature. Dissolution was complete in 15 min. The acidified solution was extracted with ethyl acetate. The extract was washed with aqueous sodium bicarbonate, dried and evaporated. The residue in ethyl acetate (5 ml) was chromatographed on alumina (18×2 cm) in benzene. Fractional elution with benzene-methanol (200:1) gave, after 300 ml of forerun, gibberellin C methyl ester (Ib, about 13 mg in 150 ml of eluate), m.p. 220~223°C, identified by mixed m.p. determination and infrared measurement.

6) K. Mori, T. Ogawa, M. Matsui and Y. Sumiki, unpublished.

7) A. Bowers, T.G. Halsall, E.R.H. Jones and A.J. Lemin, *J. Chem. Soc.*, **1953**, 2548.

Further elution (500 ml) afforded the starting 2 (eq) - hydroxy-epimer (IIb, about 150 mg).

Gibberellin C (Ia) from its Methyl Ester (Ib) by Acid Hydrolysis. Gibberellin C methyl ester (Ib, 50 mg) in methanol (2 ml) was refluxed with dilute hydrochloric acid (1:4, 10 ml) for 3.5 hrs. The mixture was extracted with ethyl acetate. The extract was dried over sodium sulfate and concentrated. Crystallization of the residue from ethyl acetate-petroleum ether afforded prisms (25 mg) of gibberellin C (Ia), m.p. 233~237°C. Recrystallization raised the m.p. to 248~250°C. $\nu_{\max}$ 3520, 3470, 3330, 1758, 1718, 1700, 1640 cm^{-1} . This was identified by mixed m.p. determination and infrared measurement.

Gibberellin C Methyl Ester 8-Dithioethyleneketal (VIa). Gibberellin C methyl ester (Ib, 4.5 g) in chloroform (80 ml) was treated with ethanedithiol (4.5 ml) and boron trifluoride etherate (4.5 ml). After having been left to stand overnight at room temperature, the mixture was poured into ice-water. The chloroform layer was separated, washed with brine, dried and evaporated. The residue crystallized when triturated with ether. Yield, 4.7 g (87%). Needles from ethyl acetate-petroleum ether, m.p. 222~223°C. *Anal.* Found: C, 59.94; H, 6.81. Calcd. for $\text{C}_{22}\text{H}_{30}\text{O}_5\text{S}_2$: C, 60.24; H, 6.67%. $\nu_{\max}$ 3484, 1752, 1732 cm^{-1} .

Desoxogibberellin C Methyl Ester (VIIb). The above described thioketal (VIa, 4.6 g) in dioxane (200 ml) was boiled with Raney nickel W-7 (35 g) for 12 hrs. The catalyst was filtered off and washed with dioxane. The combined filtrate and washings were concentrated in vacuo. Trituration of the residue with petroleum ether afforded 2.7 g (75%) of crystals. Prisms from ethyl acetate-petroleum ether, m.p. 159~160°C. *Anal.* Found: C, 69.25; H, 7.93. Calcd. for $\text{C}_{20}\text{H}_{28}\text{O}_5$: C, 68.94; H, 8.10%. $\nu_{\max}$ 3540, 1758, 1728 cm^{-1} .

Acetate (VIIc) of Desoxogibberellin C Methyl Ester. (A) The above described ester (VIIb, 80 mg) in pyridine (1 ml) was acetylated with acetic anhydride (1 ml). Subsequent treatments afforded 80 mg (90%) of crystals. Needles from ethyl acetate-petroleum ether, m.p. 134~135°C. *Anal.* Found: C, 68.04; H, 7.69. Calcd. for $\text{C}_{22}\text{H}_{30}\text{O}_6$: C, 67.67; H, 7.74%. $\nu_{\max}$ 1765, 1738, 1722 cm^{-1} . (B) Gibberellin C methyl ester acetate (Ic, 900 mg) in chloroform (15 ml) was treated with boron trifluoride etherate (1 ml) and ethanedithiol (1 ml). Subsequent treatments as in

the preparation of the thioketal (VIa) afforded an oil. This oil in benzene-petroleum ether (1:1, 10 ml) was chromatographed on alumina (20×1.8 cm) in benzene-petroleum ether (1:1). The column was washed with benzene-petroleum ether (1:1, 500 ml) to remove ethanedithiol. Elution with benzene-methanol (150:1, 400 ml) gave the desired thioketal (VIb) as an oil. This was boiled with Raney nickel W-7 (15 g) in dioxane (100 ml) for 13 hrs. Subsequent treatments as described before afforded 700 mg (87%) of needles, which was identical (mixed m.p. and infrared spectrum) with the acetate obtained by acetylation as described above.

Oxidation of Desoxogibberellin C Methyl Ester (VIIb). To a solution of desoxogibberellin C methyl ester (VIIb, 800 mg) in acetone (15 ml) was added 0.6 ml of Jones' reagent at 5°C. The mixture was allowed to stand at room temperature for an hour and then diluted with water (30 ml). Precipitated crystals of methyl 1-carboxy-1,7-dimethyl-2-oxo-4a-hydroxy-7a-gibbane-10-carboxylate 1→4a-lactone (VIII) were collected. Yield, 750 mg (94%). Needles from ethyl acetate-petroleum ether, m.p. 132~133°C. *Anal.* Found: C, 69.57; H, 7.19. Calcd. for $\text{C}_{20}\text{H}_{28}\text{O}_6$: C, 69.34; H, 7.57%. $\nu_{\max}$ 1770, 1732, 1714 cm^{-1} .

Desoxogibberellin C (VIIa). Desoxogibberellin C methyl ester (VIIb, 500 mg) was boiled with dilute hydrochloric acid (1:5, 30 ml) for 4 hrs. The mixture was extracted with ethyl acetate. The extract was separated into neutral (250 mg) and acidic (150 mg) fractions by extraction with sodium bicarbonate and recovery. The crystalline neutral fraction was the recovered starting material. The acidic fraction in ethyl acetate (3 ml) was chromatographed on silicic acid (14×1 cm). Elution with chloroform-ethyl acetate (4:1) afforded 5 mg of crystals. Prisms from ethyl acetate-petroleum ether, m.p. 205~210°C (dec.). *Anal.* Found: C, 70.48; H, 7.75. (1.016 mg Subst.; CO_2 , 2.624 mg; H_2O , 0.704 mg). Calcd. for $\text{C}_{20}\text{H}_{28}\text{O}_5$: C, 68.24; H, 7.84%. $\nu_{\max}$ 3490, 1750, 1728 cm^{-1} .

Methyl 1,7-Dimethyl-2 β -hydroxy-7 α -gibb-4a (4b)-ene-1,10-dicarboxylate (X). The hydroxy keto diester (Va, 2.0 g) in chloroform (30 ml) was treated with ethanedithiol (2 ml) and boron trifluoride etherate (2 ml). Subsequent treatments including chromatography on alumina as described in the preparation of the thioketal (VIb) afforded the oily thioketal (IX). This was desulfurized by boiling with Raney nickel W-7 (15 g) in dioxane (150 ml). Subsequent treatments

as usual gave 0.75 g (39%) of crystals (X). Prisms from ethyl acetate-petroleum ether, m.p. 136~137°C. *Anal.* Found: C, 69.80; H, 8.20. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34%. $\nu_{\max}$ 3508, 1730, 1708 cm^{-1}

Methanolysis of Desoxogibberellin C Methyl Ester (VIIb). A solution of the ester (VIIb, 600 mg) in methanol (30 ml) was treated with dry hydrogen chloride as described in the preparation of the diester (IIIa). The product in a small amount of ethyl acetate was chromatographed on alumina (10×1.5 cm) in benzene. Fractional elution with benzene-methanol (200:1) gave methyl 1,7-dimethyl-2 β -hydroxy-7 α -gibb-4 α (4b)-ene-1,10-dicarboxylate (X, 150 mg), identified by mixed m.p. determination and infrared measurement. Further elution recovered a small amount of the starting material (VIIb, 20 mg).

Relactonization of the Desoxo Diester (X) to Desoxogibberellin C Methyl Ester (VIIb). A mixture of the desoxo ester (X, 100 mg), acetic acid (8 ml), water (9 ml) and concd. sulfuric acid (3 ml) was refluxed for 5 hrs. The mixture was extracted with ethyl acetate and the extract washed with water, dried, treated with dried ethereal diazomethane and evaporated. The product was chromatographed on alumina (8×1 cm) in benzene. Elution with benzene-methanol (200:1) gave about 30 mg of intractable gum and then about 10 mg of lactonic fraction (infrared measurement). About 3 mg of crystals were obtained, of which infrared spectrum was identical with that of desoxogibberellin C methyl ester (VIIb).

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