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Analytical Studies on the Active Constituents in Crude Drugs. V.[1] The Structure of Sennoside G, a New Glucoside from Senna[2]

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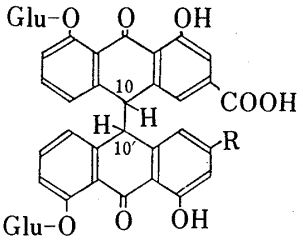
A new glucoside, sennoside G, was isolated from the leaves of *Cassia angustifolia* (Senna) in crystalline form and shown by chemical and physical means to be the optical antipode of sennoside A with respect to the sennidin moiety. The optical rotatory dispersion spectrum of sennidin G showed a pattern exactly opposite to that of sennidin A. Sennosides A, B and G were isomerized to each other reversibly, and were oxidized to give 8-glucosylrhein.

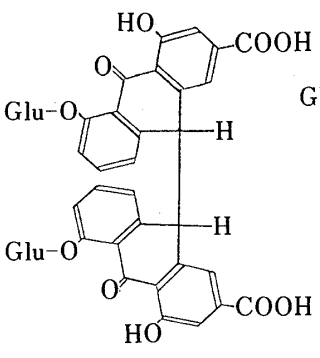
Keywords——Sennae Folium; sennoside G; isolation; determination; high performance liquid chromatography; isomerization; oxidation; reduction

Senna is a popular purgative crude drug, and its constituents have been studied by several groups of workers. Stoll and his co-workers[3] isolated sennosides A and B as the main active constituents, and determined their structures in 1950. Sennosides C and D were also isolated from Senna by Lemli *et al.*,[4] and the structure of sennoside C was determined by Schmid *et al.*[5] in 1965. Recently Oshio *et al.*[6] reported the isolation of sennosides E and F from Rhubarb.

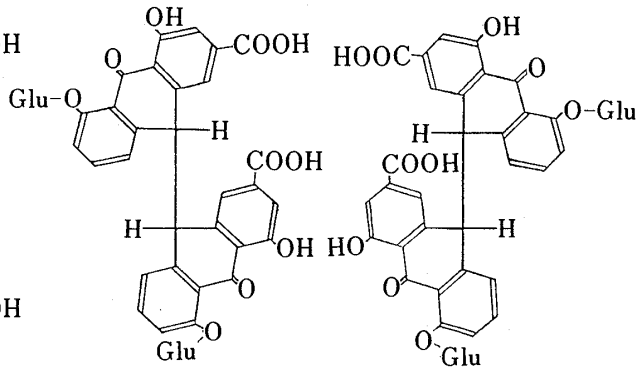
As shown in Chart 1, sennosides are glucosides possessing two moles of glucose, and are easily hydrolyzed to give sennidins. These sennidins possess at positions 10 and 10′ two asym-



sennoside A : R=COOH
sennoside B : R=COOH
sennoside C : R=CH_2OH
sennoside D : R=CH_2OH



sennoside B



sennoside A and its optical antipode

Chart 1

metric carbons which give rise to 3 or 4 isomers in the case of sennoside A or sennoside C, respectively. Therefore, further unknown sennosides may exist in Senna in addition to sennosides A, B, C and D.

In the previous paper[1] dealing with analytical studies on the active constituents in Senna, a new sennoside different from sennosides A-F was isolated, and named sennoside G.

The present investigation was undertaken to elucidate the structure of sennoside G. The isolation method for sennoside G was also investigated and the use of bone charcoal was found to be effective.

Experimental

Materials——Bone charcoal was purchased from Wako Pure Chem. Ind. Ltd. Diethyl aminoethyl (DEAE)-cellulose was obtained from Seikagaku Kogyo Co., Ltd. Sennae Folium pulv. (JP) and Sennae Folium (JP) were obtained from Japan Powder Co., Ltd. Glucostat was obtained from Fujisawa Medical Supply Co., Ltd. Other chemicals were of guaranteed reagent grade.

High Performance Liquid Chromatography (HPLC)——A Shimadzu high performance liquid chromatograph (Model PRP-1) equipped with a variable wavelength spectromonitor (Model SPD-1) set at 254 nm was used.

The operating conditions were as follows. (A) column, Permaphase ODS, 3 mm i.d. × 50 cm (Umetani glass column [GCT-03], dry-packed with an Umetani auto dry-packer); column temperature, room temp.; mobile phase, 2% dioxane/pH 2.2 Britton–Robinson buffer; flow rate, 1.2 ml/min; sample size, 5 μl; detector, ultra violet (UV) 254 nm; sensitivity, 0.08 Aufs. (B) column, Lichrosorb RP-18, 4 mm i.d. × 15 cm; mobile phase, 30% MeOH/pH 3.4 succinate-borate buffer.

Isolation of Sennoside G——Preparation of Sennoside Ca:[7] About 100 g of Sennae Folium pulv. or finely powdered Sennae Folium was extracted with two 700 ml portions of 70% methanol. The extract was concentrated *in vacuo* to 150 ml at below 35°C, adjusted to pH 9.0 with trimethylamine, and then washed three times with butanol saturated with water. The brown residue was acidified to pH 3.0 with 2 N HCl, then extracted with three 300 ml portions of butanol saturated with water. After being washed with water, the extract was evaporated to dryness *in vacuo* at below 50°C; 4 g of yellow-brown powder was obtained. This powder was washed with propyl alcohol, then dissolved in 100 ml of methanol containing 2 ml of triethylamine. $CaCl_2$ (10 g in 100 ml of MeOH) was added to this solution. About 1 g of sennoside Ca was obtained as a red precipitate.

Treatment with Bone Charcoal: Sennoside Ca (50 g) dissolved in 0.1 N NaOH (500 ml) was treated with bone charcoal (1.5 kg) in order to exclude impurities. After filtration, the filtrate was acidified with 2 N HCl (300 ml), then 30 g of bone charcoal was added. The bone charcoal was collected by filtration and extracted with 2% triethylamine (500 ml). The triethylamine extract was rinsed to neutrality with ether (300 ml × 3). The water layer was concentrated *in vacuo* to a small volume at below 50°C. About 30% of sennoside G was recovered through the bone charcoal treatment.

DEAE-cellulose Column Chromatography: The above solution was applied to a DEAE-cellulose column (Cl^- type, 3 × 28 cm), and eluted with 0.15 M NaCl. The sennoside G fraction was collected (2500 ml), acidified with HCl and adsorbed on bone charcoal (10 g). In the same manner as described above, the bone charcoal was extracted with 500 ml of 2% triethylamine. This extract was rinsed with ether (300 ml × 3). The water layer was concentrated *in vacuo* to a small volume at below 50°C, then allowed to crystallize at 0°C after acidification with HCl. Recrystallization from acetone–H_2O (7: 3) provided 300 mg of sennoside G, yellow plates, mp 162—176°C (dec.). $[\alpha]_D^{28}$ −110° (c=0.1, 70% dioxane). *Anal.* Calcd for $C_{42}H_{38}O_{20}$: C, 58.47; H, 4.44. Found: C, 58.37; H, 4.75. UV $\lambda_{max}^{0.5\%\ NaHCO_3}$ nm: 269, 318, 348.

Thin Layer Chromatography (TLC)——Condition (A) for sennosides and sennidins: pre-coated Kieselgel 60 F_{254} (Merck), AcOEt–1-propanol–H_2O (ratio of 4: 4: 3). Condition (B) for sugar: pre-coated Kieselgel 60 (Merck), AcOEt–isopropanol–H_2O (195: 70: 35).

Hydrolysis of Sennoside G——Sennoside G (16 mg) suspended in 20 ml of 1 N HCl solution was heated at 100°C for 90 min. The reaction mixture was extracted with 30 ml of AcOEt. After being rinsed with water, the AcOEt layer was evaporated to dryness to afford a yellow residue (sennidin G), which was subjected to TLC [condition (A)], infrared (IR) spectroscopy, and optical rotatory dispersion (ORD) and optical rotational analyses. On the other hand, the water layer was subjected to TLC [condition (B)] for identification of the sugar.

Measurement of the specific optical rotation of sennidin G: The above yellow residue was dissolved in 10 ml of 70% dioxane, then the optical rotation was measured. $[\alpha]_D^{28}$ *ca.* −150° (c=0.1, 70% dioxane).

Quantitative Analysis of Glucose from Sennoside G——Sennoside G (3 mg) was suspended in 20 ml of 1 N HCl solution, and heated at 100°C for 90 min. The precipitated yellow aglycone was filtered off, and the filtrate was neutralized with 1 N NaOH, then this colorless solution was concentrated to exactly 20 ml. The

content of glucose of this solution was determined by the use of Glucostat.

Reduction of Sennoside G with $Na_2S_2O_4$——Sennoside G (2 mg) was dissolved in 5 ml of 0.1 N $NaHCO_3$ solution, then 250 mg of $Na_2S_2O_4$ was added and the mixture was heated on a boiling water bath for 10 min. After cooling, the reaction mixture was subjected to HPLC [condition (A)]. Sennoside B was also reduced in the same manner.

Isomerization among Sennosides A, B and G——Each sennoside (5 mg) was dissolved in 30 ml of 0.01% $NaHCO_3$ and heated at 85°C for 4 h under bubbling with N_2 gas. After cooling to room temp., the reaction mixtures were injected into the HPLC column [condition (A)].

Oxidation of Sennoside G with H_2O_2——Sennoside G (0.2 mg) was dissolved in 1 ml of 0.5% $NaHCO_3$ and 0.1 ml of 3.5% H_2O_2 was added. The mixture was heated on a boiling water bath for 15 min, then allowed to cool to room temp. An aliquot of the reaction mixture was injected into the HPLC column [condition (B)].

Determination of Sennoside G Content in Senna Leaf——Finely powdered Senna leaves (200 mg) were extracted with 10 ml of water for 20 min on a reciprocal shaker. After filtration, 5 μl of the extract was injected into the HPLC column [condition (A)]. The calculation of sennoside G content was performed by comparing the peak height on HPLC with that of a standard solution. The content of sennoside G in Senna was about 0.17%.

Standard solution: 0.05% sennoside G dissolved in 70% dioxane.

Results and Discussion

Isolation of Sennoside G from Senna

The purification procedure for sennoside G consisted of three steps; 1) preparation of sennoside Ca, 2) treatment with bone charcoal, 3) DEAE-cellulose column chromatography.

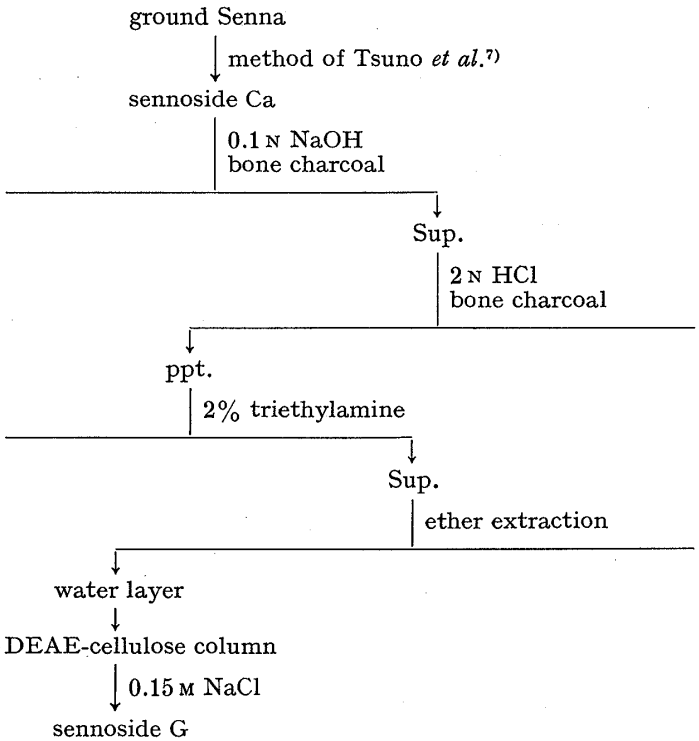


Chart 2. Isolation of Sennoside G

First of all, finely ground Senna was treated by the method of Tsuno *et al.*[7] to afford partially purified "Sennoside Ca." Bone charcoal was added to the sennoside Ca dissolved in 0.1 N NaOH. After filtration, the filtrate was acidified with HCl and then a small amount of bone charcoal was added, in order to adsorb and concentrate sennosides. After filtration, the sennosides were reextracted with 2% triethylamine from the bone charcoal. This treat-

ment was based on the following facts. i) Sennosides are adsorbed on bone charcoal easily in acidic solution, but with difficulty in alkaline solution. ii) There is a difference in the adsorption activity of sennosides on bone charcoal. The order of adsorption strength was as follows: sennoside B> sennoside A > sennoside G. In this step, sennoside G was fairly well separated from other sennosides.

The triethylamine solution was shaken with ether in order to remove excess triethylamine. After concentration to a small volume *in vacuo*, the water layer was subjected to DEAE-cellulose column chromatography. The sennoside G fraction was collected and recrystallized from 70% acetone.

The purity of sennoside G was examined by HPLC [condition (A)] as shown in Fig. 1. The total yield of sennoside G was about 0.006% from Sennae Folium pulv. The content of sennosides A, B and G in Sennae Folium were as follows; sennoside A 0.458%, sennoside B 0.838%, sennoside G 0.214%.[1)]

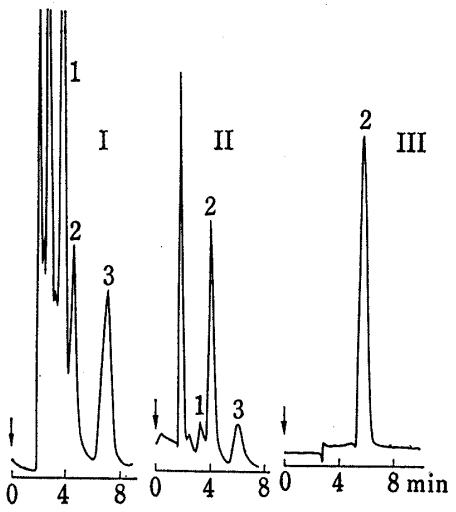


Fig. 1. Purity of Sennoside G

I: Sennoside Ca II: Treatment with Bone Charcoal III: Treatment with DEAE-cellulose column 1: sennoside B 2: sennoside G 3: sennoside A HPLC conditions: Permaphase ODS (3 mm i.d. × 50 cm), room temp.; mobile phase, 2% dioxane/pH 2.2 buffer; flow rate, 1.2 ml/min; detector, UV 254 nm

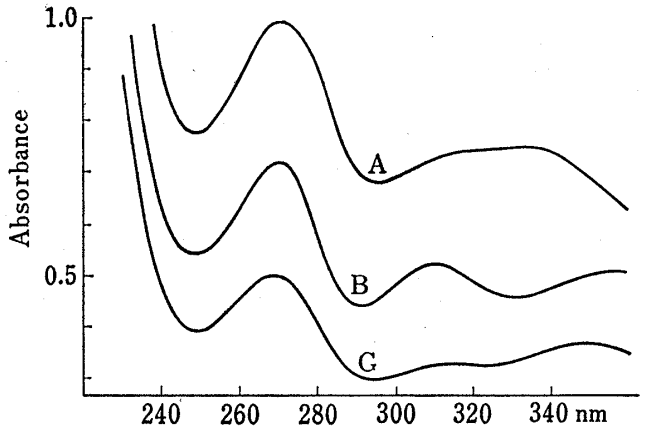


Fig. 2. UV Spectra of Sennoside A, B and G in 0.5% $NaHCO_3$ Solution

Characterization and Identification of Sennoside G

The *Rf* value of sennoside G on TLC was 0.27, almost the same as that of sennoside B (0.26). The ultraviolet spectrum of sennoside G showed a pattern of absorption rather similar to that of sennoside A or B (Fig. 2). The IR spectrum of sennoside G was essentially identical with those of sennosides A and B (Fig. 3). The ORD spectrum showed a pattern substantially opposite to that of sennoside A (Fig. 4).

Sennoside G was hydrolyzed to give one mole of aglycone, sennidin G, and two moles of glucose on being heated in mineral acid solution. The identification of glucose was performed by TLC [condition (B)], and the glucose contents were determined by the glucose oxidase method (Glucostat). The *Rf* value of seennidin G was exactly the eame as that of sennidin A or B [0.16, condition (A)]. The IR spectrum of sennidin G was also essentially identical with those of sennidins A and B, and its trimethylsilyl derivative showed the same fragmentation pattern as those of sennidins A and B in the mass spectrum (MS). As shown in Table I, the specific optical rotation of sennidin G was about −150°C, which was nearly opposite to that of sennidin A (*ca.* +180°).[3)] Furthermore, in the ORD spectrum, sennidin G showed a Cotton

Table I. Specific Optical Rotation of Sennosides and Sennidins

Sennoside A[3] $[\alpha]_D^{20}$ $-24°$ ($c=0.2$)	Sennidin A[3] $[\alpha]_D^{20}$ *ca.* $+180°$ ($c=0.1$)
Sennoside B[3] $[\alpha]_D^{20}$ $-67°$ ($c=0.4$)	Sennidin B[3] $[\alpha]_D^{20}$ $0°$ ($c=0.1$)
Sennoside G $[\alpha]_D^{28}$ $-110°$ ($c=0.1$)	Sennidin G $[\alpha]_D^{28}$ *ca.* $-150°$ ($c=0.1$)

Solvent: dioxane–H_2O (7: 3).

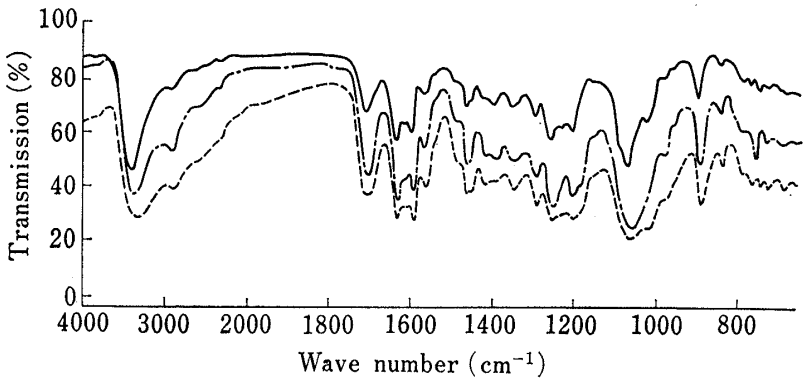


Fig. 3. IR Spectra of Sennosides A, B and G (KBr disk)
——: sennoside A, —·—: sennoside B, ----: sennoside G.

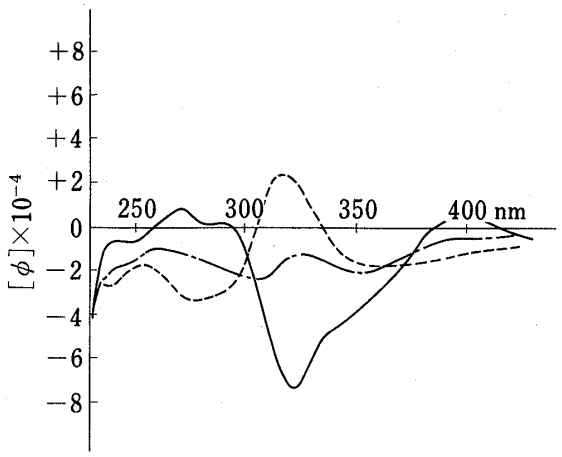


Fig. 4. ORD Spectra of Sennosides A, B and G in Dioxane–H_2O (7: 3)
——: sennoside A, —·—: sennoside B, ----: sennoside G.

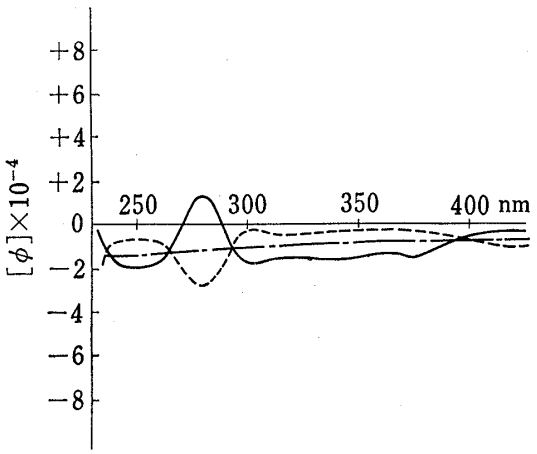


Fig. 5. ORD Spectra of Sennidins A, B and G in Dioxane–H_2O (7: 3)
——: sennidin A, —·—: sennidin B, ----: sennidin G.

effect exactly opposite to that of sennidin A at 280 nm. Sennidin B did not show any optical activity because it is a *meso* form (Fig. 5).

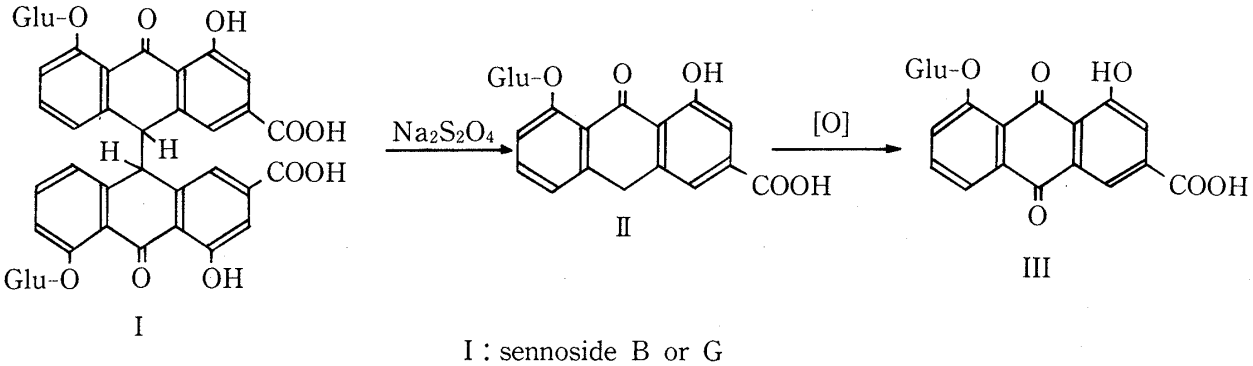
It seems clear that sennidin G is the optical antipode of sennidin A on the basis of its optical rotation, IR, MS and ORD properties.

When sennoside G was reduced with sodium hydrosulfite, 8-glucosylrhein anthrone and its oxidized form, 8-glucosylrhein, were detected by HPLC. Similarly, sennoside B afforded 8-glucosylrhein anthrone and 8-glucosylrhein upon sodium hydrosulfite reducton, and the HPLC pattern of the reduced sennoside G was almost the same as that of sennoside B (Chart 3).

Upon oxidation with H_2O_2, sennoside G afforded only one compound, 8-glucosylrhein, as determined by HPLC (Chart 4).

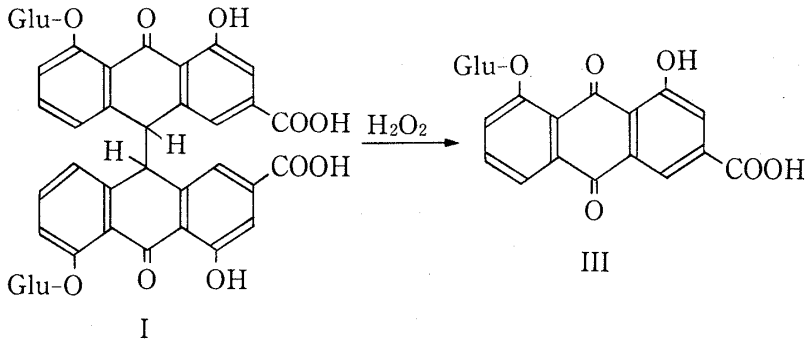
Sennosides A, B and G were able to isomerize slowly to each other in sodium bicarbonate

solution at 80°C. Isomerization from A to B, from B to A and G, and from G to B could be observed on HPLC. During the isomerization study, the stability of each sennoside was also observed. The order of stability was as follows: sennoside B> sennoside A> sennoside G. Interestingly enough, this is the same order as that of ease of dsorption on bone charcoal, and also the same order as that of their contents in Sennae Folium.[1)]



I : sennoside B or G
II : 8-glucosylrhein anthrone
III : 8-glucosylrhein

Chart 3



I : sennoside G
III : 8-glucosylrhein

Chart 4

On the basis of the above data, it is now confirmed that sennoside G is the optical antipode of sennoside A with respect to the sennidin moiety. Sennoside G might be the same compound as sennoside III isolated by Hietala *et al.*,[8)] in view of the results of their reduction and oxidation experiments.

Two further so far undetected compounds corresponding to sennoside C and D may remain to be found as mentioned at the beginning of this report.

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References and Notes

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