Summary

The structure of hasubananine was reexamined and the complete constitution (IIc) including the absolute stereostructure was presented. The structure of homostephanoline was also investigated and three possible structure for this alkaloid were shown in formulae (XIIa), (XIIb), and (XIIc). These two alkaloids have a novel skeleton (XI) which has not been known hitherto in the natural sources, for which the name "hasubanan" was proposed.

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73. Tameto Okanishi, Akira Akahori, and Fumio Yasuda: Studies on the Steroidal Components of Domestic Plants. XLVII.*1 Constituents of the Stem of Smilax sieboldi Miq. (1). The Structure of Laxogenin.

(Shionogi Research Laboratory, Shionogi & Co., Ltd.*)

Smilax sieboldi Miq. is a climbing shrub of the Liliaceae family native to Japan, Korea, and China. It is well known that the various species of Smilax, native to Middle and South America, are the better plant source of smilagenin or sarsasapogenin, but the steroidal constituents of the oriental plants belonging to the same genus have not yet been reported, except diosgenin from S. china L.*1 Recently we obtained a new steroidal sapogenin together with tigogenin (25α,5α-spirostan-3β-ol) and neotigogenin (25α,5α-spirostan-3β-ol) from the stem of this plant.

The physical constants of the new sapogenin are as follows: C_{38}H_{54}O_{4}, m.p. 210−212°C; [α]_{D}^{25}−86.8°; IR \nu_{	ext{max}} \text{cm}^{-1}: 3490 (−OH), 1713 (C=O), 982, 920, 901, 868, 920<901 (F-ring). Acetylation of this new sapogenin with acetic anhydride and pyridine afforded a monocacetate (1b), C_{40}H_{56}O_{5}, m.p. 219−222°C; [α]_{D}^{25}−88.6°, and its infrared absorption spectra showed an acetyl band but not a hydroxyl band. Thus, this sapogenin was considered to be a 25α-monohydroxysapogenin having a six-membered ketone group. Although the known monohydroxy-monoketo-spirostanes are hecogenin (3β-hydroxy-25α,5α-spirostan-12-one) and sisalagenin (3β-hydroxy-25α,5α-spirostan-12-one), the physical constants of this new sapogenin and its acetate do not coincide with any of those sapogenins and their acetates. From these results, it is considered that this sapogenin is a new compound, and is named laxogenin according to the genus name of the parent plant.

In order to determine the position of the hydroxyl group and the ketone group in laxogenin, the following experiments were carried out.

On Huang–Minlon reduction, laxogenin gave a product, melting at 204−205°C, and this was identified as tigogenin by comparison of infrared spectrum and mixed melting point with the authentic specimen. This suggests that the structure of laxogenin is tigogenin having a six-membered ketone group.

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The sodium borohydride reduction of laxogenin afforded two diols (C_{29}H_{44}O_{4}), m.p. 242-244° (IIa) and 265-266° (Na), in a ratio of about 85:15. From comparison of the physical constants of these diols and their acetates with those of chlorogenin\(^3\) (25α,5α-spirostan-3β,6α-diol), and β-chlorogenin\(^3\) (25β,5α-spirostan-3β,6β-diol) and their acetates (Table 1), it may be concluded that the former (IIa) is β-chlorogenin and the

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<th>Genins (°C)</th>
<th>Acetates (°C)</th>
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<tr>
<td></td>
<td>m.p.</td>
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<tr>
<td>Na</td>
<td>265-266</td>
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<tr>
<td>Chlorogenin(^a)</td>
<td>271</td>
</tr>
<tr>
<td>IIa</td>
<td>242-244</td>
</tr>
<tr>
<td>β-Chlorogenin(^b)</td>
<td>249-251</td>
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other (Na) is chlorogenin. Our experience that the metal hydride reduction of C-4-ketone of the 5α-steroids gave more 6β-axial hydroxyl compound than the 6α-equatorial,4 well supports this conclusion. Furthermore the \([M]_D\) differences between these products (6-OH) and tigogenin (6-H) and their acetates, compared with those of cholestane series, also supported this conclusion (Table II). On the other hand, laxogenin acetate was reduced with sodium in ethanol and yielded two kinds of diol, two, but the main product was chlorogenin, and the yield of β-chlorogenin was very poor, in this case. This result is identical to that of Marker.

Chromic acid oxidation of laxogenin afforded a diketone (V). Admixture of this ketone with 3,6-diketone, m.p. 229~231°, prepared from diosgenin5 (Ⅵa), showed no depression of the melting point. Further, its chemical properties, analytical values, infrared absorption spectrum, and rotatory dispersion curve all agreed with those of 3,6-diketone, whereas with admixture of this ketone with 3,7-diketone6 a marked depression of the melting point occurred.

From the above-mentioned results, it may be concluded that the ketone group of laxogenin is situated at C-9 position in the molecule. In order to confirm this assumption, 3β-hydroxy-25α,5α-spirostan-6-one was synthesized from 5,6-epoxydiosgenin (Ⅹ) by the action of boron trifluoride according to the method reported by Hembest and Wrigley.7 The synthesized product was identical with laxogenin in all respects. Thus the structure of laxogenin was confirmed to be 3β-hydroxy-25α,5α-spirostan-6-one.

Since it is well known that the 5β-configuration of steroids, having a 6-ketone group easily converts to the 5α-isomer,8 there remains some doubt that this sapogenin is an isomeric substance of the 6-keto-5β-type compound, which might be formed during the process of acid hydrolysis of the saponin mixture. In order to clarify this point, the following experiments were carried out.

After acetylation of the crude saponin, obtained from Smilax sieboldi Miqu., with acetic anhydride and pyridine at room temperature overnight, the product was reduced with lithium aluminum hydride in tetrahydrofuran and followed by hydrolysis with hydrochloric acid and saponification with potassium hydroxide. The extract, thus

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obtained was chromatographed on alumina and yielded tigogenin and β-chlorogenin, whereas no sapogenin, with A/B cis juncture, could be obtained in this series of experiments. From these results and the fact that laxogenin is obtained together with tigogenin and neotigogenin, and not with the cis-sapogenin, it may be assumed that laxogenin exists naturally in plant as the saponin of the trans-type sapogenin.

Experimental

**Paper Chromatography**—Filter paper: Toyo-Roshi No. 50, 2 × 40 cm. Apparatus: Toyo-Roshi Model C. Developed with ascending method. Solvent: toluene-AcOH (50:3) (for genins), heptane-CHCl₃-AcOH (50:2:2) (for acetates). Color reagent: 1% cinnamic aldehyde in EtOH and 25 g. of ShCl in 5 ml. of nitrobenzene. RF values of the standard sapogenins: diosgenin 0.90, tigogenin 0.90.

**Extraction of the Crude Sapogenins**—The stems were collected at Ina, Nagano Pref. in June, air dried and powdered. The powder (25.5 kg.) was extracted 4 times for 6 hr. with 95% MeOH under reflux. The MeOH solution was condensed to 1.5 L., refluxed with 250 ml. of conc. HCl for 5 hr., then 1 L. of water added and extracted with 1 L. of benzene. After removal of solvent, the extract was saponified with 5% KOH-MeOH and extracted with ether. From this solution 71 g. of greenish black viscous substance (RF 0.98 (purple), 0.91, 0.69, 0.45, 0.26) was obtained.

**Isolation of Tigogenin and Neotigogenin**—After acetylation and repeated purification through alumina chromatography, the above mentioned extract was divided in 3 fractions, A, B, and C, as shown in Table III.

Table III.

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<thead>
<tr>
<th>Solvents</th>
<th>Yields (g.)</th>
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<tr>
<td>Petri. ether</td>
<td>4.8 (A)</td>
</tr>
<tr>
<td>Petri. ether-Benzene (9:1)</td>
<td>4.5 (B)</td>
</tr>
<tr>
<td>ν (7:3, 5:5)</td>
<td>2.4 (C)</td>
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Fraction A was crystallized from MeOH and yielded the crystals of m.p. 134–136°. Since this showed a purple spot, it was considered that this fraction is phytostereol, and further experiments were omitted.

Fraction B was crystallized from MeOH giving two kinds of crystals, which were identified as tigogenin acetate and neotigogenin acetate, respectively, by mixed melting points and IR spectrum.

**Laxogenin Acetate (Ib)**—Fraction C was crystallized from MeOH giving colorless prisms, m.p. 210–222°. \( [\alpha]_D^20 = -88.6^\circ (c = 0.948, \text{CHCl}_3) \). *Anal. Calcd. for C₉₀H₄₄O₄: C, 73.69; H, 9.38. Found: C, 73.34; H, 9.45. IR \( \nu_{\text{max}} \text{cm}^{-1} \): 1735, 1239 (–OAc), 1713 (six-membered ketone), 982, 920, 898, 864 (F-ring; 920 < 898).*

**Laxogenin (Ia)**—After saponification of 125 mg. of laxogenin acetate (Ib) with 1.0 g. of KOH in small amount of H₂O. The product was extracted with ether to yield 107 mg. of the crude sapogenin, which on recrystallization from hexane-acetone mixture (1:1) yielded 54 mg. of white needles, m.p. 210–212°, \( [\alpha]_D^20 = -86.3^\circ (c = 1.047, \text{CHCl}_3) \). *Anal. Calcd. for C₂₇H₂₉O₂: C, 75.31; H, 9.83. Found: C, 75.16; H, 9.82. IR \( \nu_{\text{max}} \text{cm}^{-1} \): 3490 (–OH), 1713 (six-membered ketone), 982, 920, 901, 868 (F-ring, 920 < 901).*

**Huang-Millon Reduction of Laxogenin**—Laxogenin (Ia, 50 mg.) was refluxed with 2 ml. of triethylene glycol, 0.3 ml. of 80% hydrazine hydrate, and 0.1 g. of KOH for 30 min. at 130–140°. The condenser was removed, the temperature of the solution was permitted to rise to 190–200°, and refluxing was continued for 2 hr. The crude reduction product, precipitated by addition of water, was dissolved in ether. The ether solution was washed with water, and after drying, ether was evaporated to yield 46 mg. of crystals of m.p. 193–203°. This was recrystallized from MeOH to give 7.0 mg. of crystals (E), m.p. 204–205°, \( [\alpha]_D^20 = -65.9^\circ (c = 1.077, \text{CHCl}_3) \). No depression of melting point was shown on admixture with tigogenin and the IR spectra of these substances were identical.

**Sodium Borohydride Reduction of Laxogenin Acetate (Ib)**—A mixture of 400 mg. of laxogenin acetate (Ib) in 80 ml. of absolute EtOH and 100 mg. of NaBH₄ in 10 ml. of absolute EtOH was refluxed for 2 hr. The reaction mixture was poured into water and extracted with ether. The ether solution washed with water, dried, and evaporated to yield 376 mg. of crystals, m.p. 235–240°. This was submitted to chromatography on Alumina and yielded 2 fractions. RF 0.56 (D) 302 mg., and RF 0.47 (E) 50 mg. Fraction D was recrystallized from acetone to give the crystals (Ila), m.p. 242–244°. This was identified as β-chlorogenin by mixed melting point and IR spectrum. \( [\alpha]_D^20 = -74.8^\circ (c = 1.087, \text{CHCl}_3) \). *Anal. Calcd. for C₂₇H₂₉O₂: C, 74.95; H, 10.25. Found: C, 75.03; H, 10.34. IR \( \nu_{\text{max}} \text{cm}^{-1} \): 3480, 3340 (–OH). NMR 64 c.p.s., 48.5 c.p.s. (11 mg./0.42 ml., CHCl₃).*

**β-Chlorogenin Diacetate (Ib)**—β-Chlorogenin (100 mg.) was acetylated by usual process and yielded 100 mg. of crystals, m.p. 117–122°, recrystallization from MeOH raised the melting point to 174–175°, \( [\alpha]_D^20 = -88.5^\circ (c = 1.009, \text{CHCl}_3) \). *Anal. Calcd. for C₂₇H₂₉O₂: C, 72.06; H, 9.36. Found: C, 72.06; H, 9.35.*

**Chlorogenin Acetate (Ib)**—From the above mentioned fraction E (50 mg,) yielded 30 mg. of crystals (Ila) melting at 249–250° after recrystallization from acetone. On acetylation by the usual process this
yielded 30 mg. of the acetate, m.p. 151-152°, which was confirmed to be chlorogenin diacetate (Ib) by determination of melting point and comparison of IR spectrum with those of authentic specimen.

Sodium Reduction of Laxogenin Acetate (Ib) — A solution of 200 mg. of laxogenin acetate (Ib) dissolved in 50 ml. of absolute EtOH. 4.9 g. of metal Na was added slowly, and after the Na was completely dissolved, the reaction mixture was poured into water and extracted with ether. The ether solution was washed with water, dried over Na$_2$SO$_4$, and ether was evaporated to yield 194 mg. of white crystals. Since this product showed two spots (Rf 0.71 and 0.49) on paper chromatography, this was submitted to Alumina chromatography. The mixed solvent, benzene-CHCl$_3$(4:6-2:8) yielded 30 mg. of crystals (Rf 0.71), recrystallization from MeOH raised the melting point to 204-205°, and this was identified as laxogenin (Ia) by mixed melting point. IR $\nu_{\text{max}}$ cm$^{-1}$: 3220(=O), 1713(=C-O), 980, 918, 897, 864(F-ring). The mixed solvent, CHCl$_3$-CHCl$_3$-MeOH(9:1) yielded 149 mg. of crystals, Rf 0.49, which on crystallization from MeOH afforded 85 mg. of crystals, m.p. 265-266°. This was acetylated by the usual manner and gave 80 mg. of crude acetate, m.p. 115-117°, which afforded 34 mg. of crystals (Nb), m.p. 151-152°, by recrystallization from MeOH. Nb was identified as chlorogenin diacetate by mixed melting point and IR spectrum. $[\alpha]_D^25=+41.1$(c=1.160, CHCl$_3$). *Anal.* Calcd. for C$_9$H$_6$O$_4$: C, 72.06; H, 9.36. Found: C, 72.27; H, 9.41. IR $\nu_{\text{max}}$ cm$^{-1}$: 1740(=Oac), 981, 920, 899, 863(F-ring).

Chlorogenin (IVA)—The above mentioned acetate (Na) (29 mg.) was dissolved in 10 ml of MeOH, and 0.5 g. KOH, in small amount of water, was added. After refluxing for 2 hr., the reaction mixture was extracted with ether. This ether solution yielded 24 mg. of crystals, which gave 12 mg. of chlorogenin (Na), m.p. 222-224°, by recrystallization from MeOH. $[\alpha]_D^25=+48.7$(c=0.663, CHCl$_3$). *Anal.* Calcd. for C$_9$H$_6$O$_4$: C, 74.95; H, 10.25. Found: C, 75.21; H, 10.23. IR $\nu_{\text{max}}$ cm$^{-1}$: 3255(=O), 980, 918, 899, 865(F-ring).

Chromium Trioxide Oxidation of Laxogenin (Ia)—To a solution of 90 mg. of laxogenin dissolved in 20 ml of AcOH, a solution of 100 mg. of Cr$_2$O$_7^{2-}$ in 5 ml of 90% AcOH was added slowly with stirring, and the mixture was allowed to stand at room temperature for 30 min. and processed in the usual manner to yield 82 mg. of crude diketone, m.p. 150-155°. Recrystallization from AcOH gave 79 mg. of crystals (V), m.p. 229-231°, as colorless needles. This was identical with 3,6-diketone in all respects, whereas admixture of this ketone with 3,7-diketone, a marked depression of the melting point occurred.

250-Spirost-4-ene-3,6-dione (X)—Dioxygenin (Via) (2.0 g.) in 100 ml of AcOH was oxidized with 2.0 g. of Cr$_2$O$_7^{2-}$ in 40 ml. of 80% AcOH for 1 hr. at room temperature. The reaction mixture was poured into water and extracted with ether. After washing with 2N Na$_2$CO$_3$, the ether was removed to yield 1.3 g. of yellowish-white crystals, which were submitted to chromatography through Alumina and yielded 750 mg. of eluate from the mixed solvent, petr. ether-benzene (2:8) and CHCl$_3$. This was recrystallized from EtOH to yield 40 mg. of the light yellowish crystals (X), m.p. 194-195°, Rf 0.43. $[\alpha]_D^25=-112.8$(±2) (c=1.067, CHCl$_3$). *Anal.* Calcd. for C$_{12}$H$_8$O$_4$: C, 76.02; H, 8.98. Found: C, 75.87; H, 9.15. UV $\lambda_{\text{max}}$ nm (e): 249(10048), IR $\nu_{\text{max}}$ cm$^{-1}$: 1692(C=O), 1616(double bond).

5a,250-Spirostane-3,6-dione (V)—A solution of 235 mg. of X in 30 ml of AcOH was heated with 1.0 g. of Zn powder for 4 hr. on a water bath. This solution after filtration, concentration, dilution with water, extraction with ether, and evaporation of the solvent furnished 191 mg. of white crystals (Rf 0.55, 0.46). Recrystallization from MeOH yielded crystals, m.p. 226-229° (15 mg.), 224-226° (7 mg.) and 221-223° (43 mg.). No depression of the melting point occurred on admixture with the 3,6-diketone, prepared from laxogenin, on CrO$_3$ oxidation. $[\alpha]_D^25=+75.1$(c=1.054, CHCl$_3$), IR $\nu_{\text{max}}$ cm$^{-1}$: 1720(=C=O). *Anal.* Calcd. for C$_{12}$H$_8$O$_4$: C, 75.66; H, 9.41. Found: C, 75.80; H, 9.52.

5a,6a-Epoxy-250-spirost-3a-ol (XI)—A solution of 2.0 g. of dioxygenin in 30 ml of CHCl$_3$ was added 14 ml of monopropyllic acid (1 ml=79.5 mg.) and allowed to stand for 48 hr. at 0-2°. After filtration, the filtrate was washed with the aqueous solution of Na$_2$HCO$_3$, dried over Na$_2$SO$_4$, and the solvent removed to yield 2.1 g. of white crystals. Recrystallization from MeOH gave 100 mg. of crystals (X) of m.p. 218-220° and 219-219°(1.3 g.). $[\alpha]_D^25=-126.7$(c=1.056, CHCl$_3$). IR $\nu_{\text{max}}$ cm$^{-1}$: 799($\alpha$-epoxy). *Anal.* Calcd. for C$_{12}$H$_8$O$_4$: C, 75.31; H, 8.83. Found: C, 75.18; H, 9.81.

3,9-Hydroxy-5a,250-spirost-6-one (XII)—A mixture of 1.3 g. of XI in 62 ml of anhydrous benzene with 2.4 ml of BF$_3$-ether complex was left at 5 min. at room temperature, dried over Na$_2$SO$_4$, the solvent removed and yielded 1.4 g of white viscous substances, m.p. 135-137°. $[\alpha]_D^25=-92.18$(c=1.018, CHCl$_3$). These were submitted to chromatography through Alumina (Woelm, 100 g.), and 423 mg. of viscous substance was obtained from the CHCl$_3$ eluate. A part (70 mg.) of this substance was dissolved in 30 ml of MeOH, and refluxed with 3 ml of conc. HCl for 1 hr., extracted with ether. Removing the solvent yielded 69 mg. of residue. Since this residue showed 2 spots (Rf 0.91, 0.55) on thin-layer chromatography (benzene-Acetone-AcOH=14:6:0.5; laxogenin Rf 0.55), this was rechromatographed on Alumina (Merck, 5 g.), and 53 mg. of eluate, showing only one spot (Rf 0.55), was obtained from the mixed solvent, benzene-CHCl$_3$(45:5-10:40). This was acetylated and recrystallized from MeOH to yield 54 mg. of acetate, m.p. 217-219°. $[\alpha]_D^25=-80$(c=0.644, CHCl$_3$). No depression of the melting point was seen by admixture with laxogenin acetate. *Anal.* Calcd. for C$_{13}$H$_{10}$O$_3$: C, 73.69; H, 9.38. Found: C, 73.54; H, 9.39.
Lithium Aluminum Hydride Reduction of the Acetylated Product of Crude Saponin Isolated from *Smilax sieboldi* Miq.—The mixture of 60 g. of crude saponin, isolated from the stem of *Smilax sieboldi* Miq., 300 ml. of pyridine and 60 ml. of Ac₂O was kept at room temperature overnight. The reaction mixture was diluted with water and extracted with ether to yield 42 g. of brownish–black viscous substance. This was dissolved in 400 ml. of abs. tetrahydrofuran and 10 g. of LiAlH₄, suspended in 200 ml. of tetrahydrofuran was added dropwise within 1 hr. The mixture was kept at room temperature for 30 min., with stirring, then heated for 1 hr. After cooling, the LiAlH₄ was decomposed by adding water, 200 ml. of 2N HCl added to dissolve the precipitate, and the solvent removed in vacuo to concentrate to about 300 ml. This was refuxed with 300 ml. of MeOH and 100 ml. of conc. HCl for 4–5 hr. After removal of MeOH, water was added and the precipitate was refuxed with 200 ml. x 3 of CHCl₃. The CHCl₃ solution was washed with alkaline solution, and the solvent was removed to yield a brownish viscous substance. This was dissolved in 100 ml. of MeOH, and refuxed with 5 g. of KOH in 30 ml. of water. To this water was added, and extracted with CHCl₃. After washing and drying, the solvent was evaporated to yield 729 mg. of brownish viscous substance (RF 0.65, 0.40). This substance was chromatographed on Alumina (Merck, 20 g.). A viscous substance (G, 106 mg.) from the mixed solvent, benzene–CHCl₃ (49:1:40:10), and 163 mg. of viscous substance (H) from CHCl₃–CHCl₃–MeOH (49:1:48:2) were obtained, respectively.

G was acetylated with 1.0 ml. of pyridine and 0.5 ml. of Ac₂O by refluxing for 5 hr., water added and extracted with ether to yield 106 mg. of extract. This was chromatographed on Alumina, and from the mixed solvent, petr. ether–benzene (40:1:10:40) yielded 34 mg. of the extract (RF 0.80). Recrystallization from MeOH gave 5 mg. of crystals of m.p. 179–187°. This was proved to be identical with tigogenin by determination of mixed melting point and comparison of IR spectrum with that of tigogenin.

H was acetylated by the usual process and yielded 260 mg. of extract (RF 0.60, 0.46). This extract was submitted to chromatography through Alumina, and from the solvents, petr. ether–benzene (20:30:10:40):benzene–benzene–CHCl₃ (49:1:45:5) yielded 104 mg. of the extract. This was dissolved in MeOH, and after concentration extracted with ether. The ether solution was washed, dried, and evaporated to yield 82 mg. of viscous substance (RF 0.35, 0.44). Rechromatography of this substance, from the mixed solvent, benzene–CHCl₃ (28:8:10:90) yielded 5 mg. of crystals, which was purified by thin-layer chromatography and recrystallized from the mixed solvent, acetone–hexane to yield 5 mg. of the crystals of m.p. 235–238°. This was proved to be identical with β–chlorogenin, m.p. 236–239°, by determination of mixed melting point and comparison of IR spectrum.

The authors express their deep gratitude to Dr. K. Takeda, Director of this Laboratory, for his helpful guidance and to Prof. Dr. S. Ohkura, Shinshu University, for collecting plant materials. Thanks are also due to the members of the analytical section of this Laboratory for microanalysis, measurement of infrared spectra and optical rotation.

**Summary**

From the aerial parts of *Smilax sieboldi* Miq, a new steroidal sapogenin, laxogenin (I), was isolated together with tigogenin and neotigogenin. The analytical values of I corresponded to formula C_{33}H_{44}O_{5} for sapogenin and C_{33}H_{44}O_{6} for the acetate. On Huang–Minlon reduction I afforded tigogenin (II), and on sodium borohydride reduction of I, β–chlorogenin (III) and chlorogenin (IV) were obtained in ratio about 85:15. The chromic acid oxidation of I afforded a diketone (V), m.p. 229–231°, which was proved to be identical with 25α–allospirostane-3,6-dione. From these results the structure of laxogenin was assumed to be 3β-hydroxy-25α,5α-spirostan-6-one and this assumption was confirmed by the synthesis of this compound from 5,6-epoxydiosgenin (X).

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