19. Shoji Shibata and Hiroshi Mitsuhashi: On the Structure of α-Hydroxysantonin.

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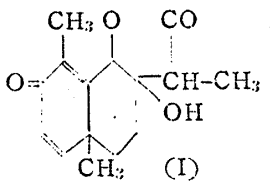
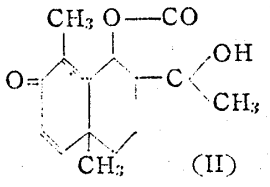
In 1897, Jaffé[1] found that a dog metabolizes *l*-santonin, $C_{15}H_{18}O_3$, converting it into a biological oxidation product, $C_{15}H_{18}O_4$, m.p. 283°, $[\alpha]_D^{20}$ −133.9° (alcohol), which was obtained from the urine and designated as α-hydroxysantonin.

The structure of α-hydroxysantonin was once examined by Asahina and Momose[2] and two alternative formulae corresponding to 6- or 11-hydroxysantonin, (I) and (II), were put forward. However, any decisive evidence for the final establishment of the structure has not been offered as yet.

On the other hand, it is already known that artemisin, m.p. 203°, $[\alpha]_D$:−84.3° (alcohol), a minor principle of *Artemisia maritima* L., is represented by 6-hydroxysantonin (I)[3].

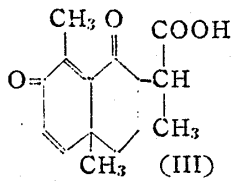


(I)

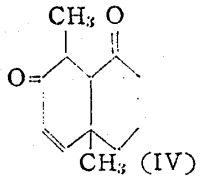


(II)

Artemisin developes a red coloration with sodium methoxide as shown by *l*-santonin, whereas α-hydroxysantonin gives only a faint yellow color. On treatment with 10% alkali, the former is converted into artemionic acid (III), whereas the latter yields a neutral diketonic compound, $C_{12}H_{16}O_2$, whose structure was described by Asahina and Momose as (IV).



(III)



(IV)

The purpose of the present paper is to describe studies carried out for establishing the structure of α-hydroxysantonin.

The hydroxyl group of α-hydroxysantonin was assigned by the former workers[2] to be tertiary by the measurement of the rate of acetylation. The 6 or 11 position would, therefore, be offered as reasonable for the hydroxyl group.

If the hydroxyl occupies the 6-position, α-hydroxysantonin should be represented as a stereoisomer of artemisin.

Regarding from the marked difference of the behaviors observed between artemisin and α-hydroxysantonin, as described above, it would scarcely be believed that both compounds are represented by the stereoisomeric relationship.

For the differentiation of the behavior of hydroxyls in 6 and 11 position, we employed an oxidative reaction with periodate which selectively attacks the structure involving vicinal hydroxyls.

The experiment showed that the cautiously neutralized sodium salt of artemisinic acid

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1) Jaffé: Z. physiol. Chem., 22, 553 (1897); Lo Monaco: Gazz. chim. ital., 27, ii, 87 (1897).
2) Y. Asahina, T. Momose: Ber., 70, 812 (1937).
3) Merck's Jahresber., 1894, 3; J. Simonsen: "The Terpenes", Vol. III, p. 312 (1952) (Cambridge Univ. Press).

consumed 1 mole of oxygen by the reaction of sodium periodate, whereas α-hydroxysantoninic acid was not affected under the same reaction condition. Any possibility of the lactone-ring closure, which might occur during the reaction, could fully be rejected by the fact that the reaction mixture was clear throughout the process. This experimental result was regarded to support the conclusion that α-hydroxysantoninic acid does not possess any hydroxyl group in α-position with respect to the hydroxyl in 5-position which engages in forming the lactone ring of α-hydroxysantonin.

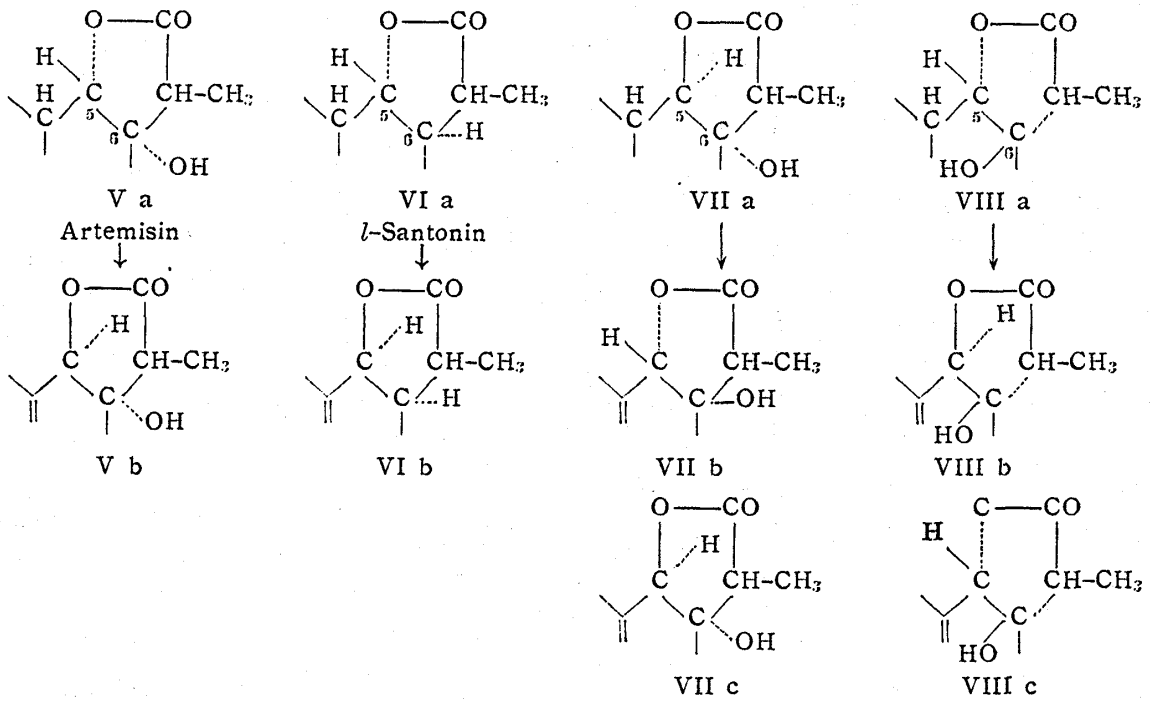
However, there remains a doubt involving the presence of *trans*-vicinal hydroxyls which are regarded to be somewhat resistant to the periodate oxidation.[4]

Further proof against this criticism might be given by the consideration on the steric relationship of santonin and desmotroposantonin.

As had been shown by the work of Huang-Minlon[5] and Balton[6], and previously confirmed by one of the present authors (M)[7], the lactone ring of santonin and artemisin is linked in a *trans*-position, and acid treatment causes inversion at C^5, forming the desmotropo compound with the *cis*-fused lactone ring.

We found that on treatment with acetic anhydride and conc. H_2SO_4, α-hydroxysantonin was converted into diacetate of desmotropo-α-hydroxysantonin in a good yield.

If the tertiary hydroxyl group of α-hydroxysantonin attaches to C^6-position in the *trans*-configuration with respect to the hydroxyl of C^5 being represented as an epimer of artemisin, the following possible configurations, (VIIa) and (VIIIa), would be considered.



When α-hydroxysantonin was converted into the corresponding desmotropo compound, it would be reasonable to expect that an analogous conversion of the asymmetric center C^5 might occur as in santonin and artemisin. It is hardly accepted, however, since the isomerization would imply the conversion of the lactone ring to the strong strained *trans*-fused form (VIIb) and (VIIIb). Assuming that no conversion of C^5 would occur in this

4) Criegee: Ann., 507, 319 (1933).
5) Huang-Minlon: J. Am. Chem. Soc., 65, 1780 (1943); *ibid.*, 70, 611 (1948).
6) D. H. R. Barton: J. Org. Chem., 15, 466 (1950).
7) H. Mitsuhashi: J. Pharm. Soc. Japan, 71, 1115 (1951).

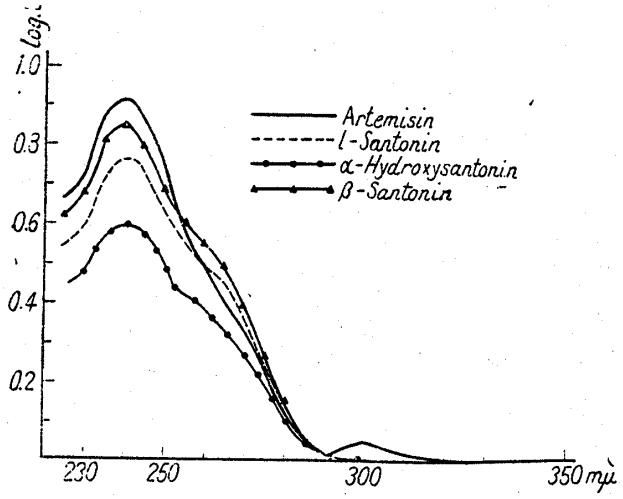


Fig. 1

isomerization, two alternative formulae, (VIIc) and (VIIIc), might be taken for desmotropo-α-hydroxysantonin.

From these possible formulae, (VIIc) must be excluded, since it is identical with desmotropoartemisin, while (VIIIc) could not be accepted, since it should give dextrorotation in analogy with the configuration at C^5-C^6 of *d*-α-desmotroposantonin.[7]

In these respects, it could very probably be concluded that α-hydroxysantonin possesses its tertiary hydroxyl in the C^{11} and be represented by the formula (II).

The ultraviolet absorption spectrum of α-hydroxysantonin was almost identical with that of *l*-santonin and artemisin (Fig. 1.).

TABLE I

Compounds	Group OH	Group lactonic : CO	Group ketonic : CO	Fig.
α-Hydroxysantonin	3.10 μ	5.60 μ	6.05 μ	2
Artemisin	2.90	5.60	6.00	3
l-Santonin	—	5.65	6.05	4
β-Santonin	—	5.65	6.05	5
l-α-Desmotroposantonin	3.05	5.80	—	6

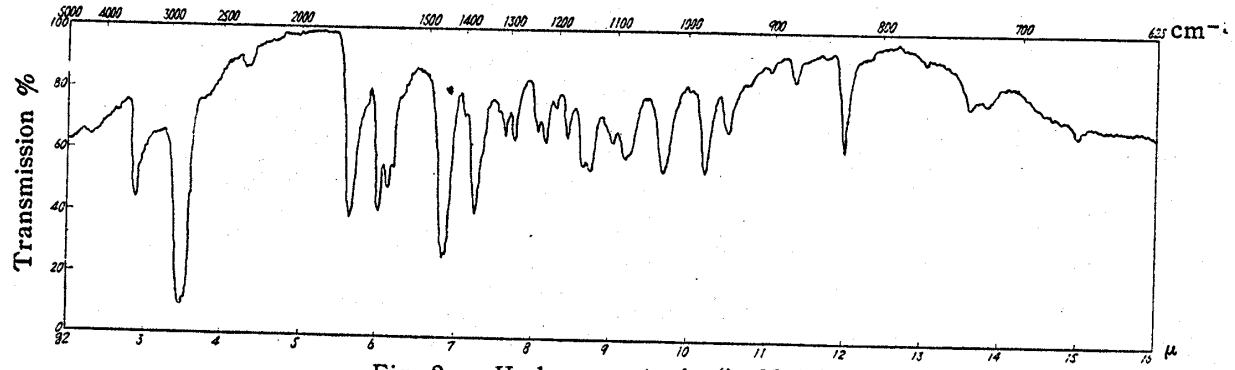


Fig. 2 α-Hydroxysantonin (in Nujol)

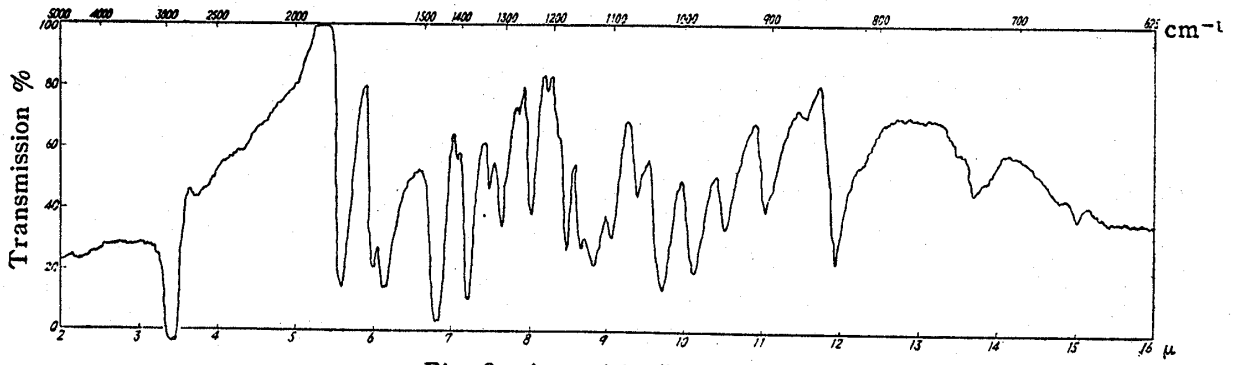
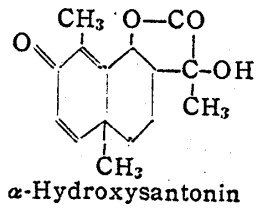
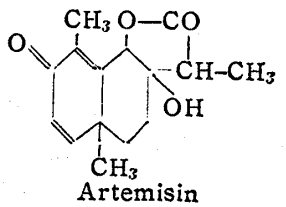


Fig. 3 Artemisin (in Nujol)



α-Hydroxysantonin



Artemisin

The result of a comparative study on the infrared absorption spectra of α-hydroxysantonin and artemisin with respect to that of *l*-santonin, β-santonin, and *l*-α-desmotroposantonin are summarized in Table I.

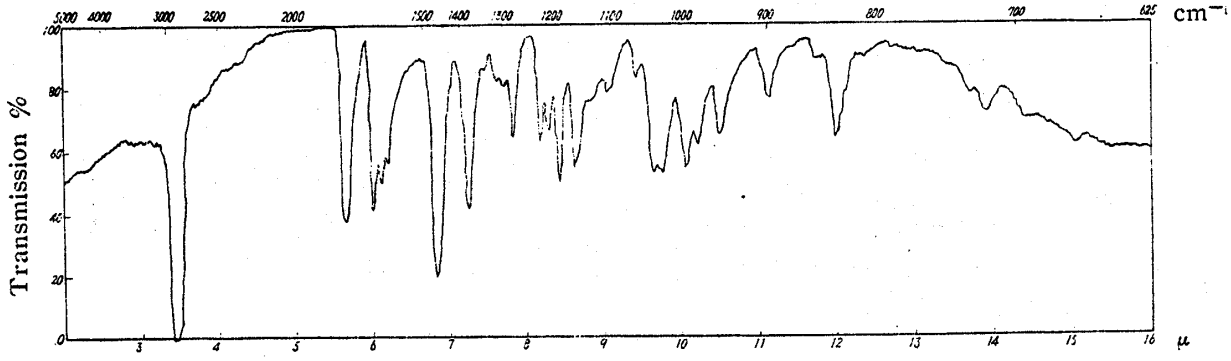


Fig. 4 *l*-Santonin (in Nujol)

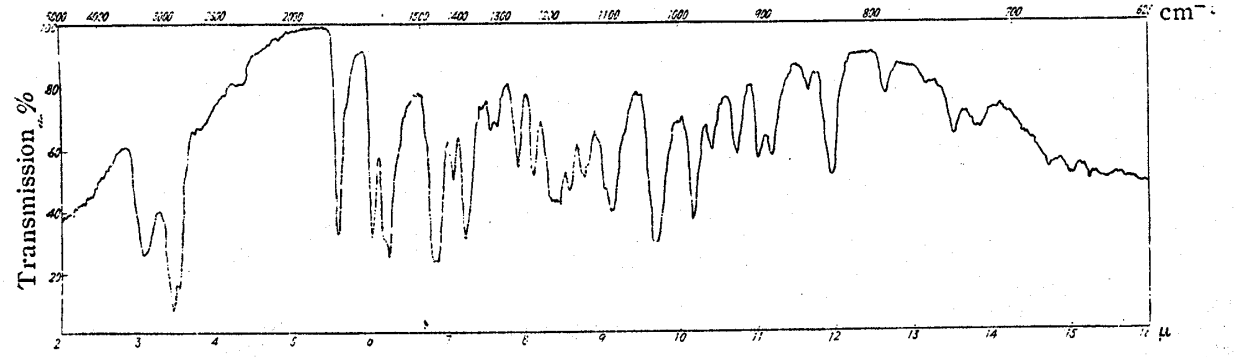


Fig. 5 β-Santonin (in Nujol)

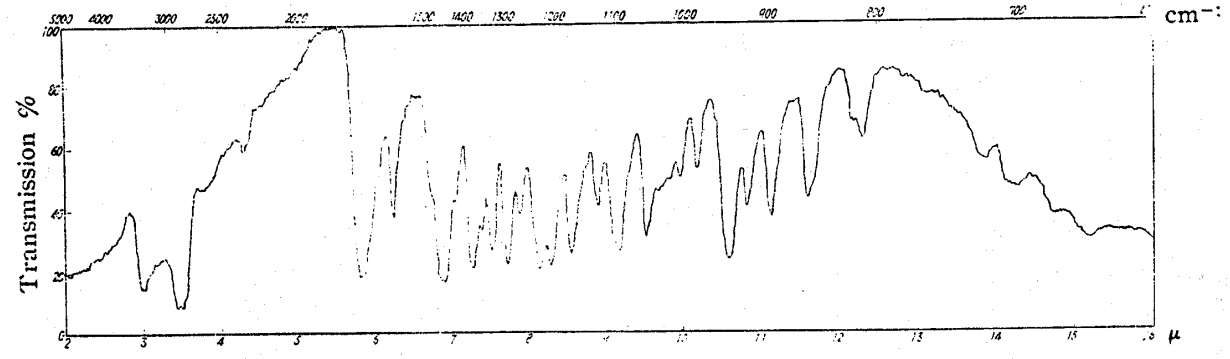
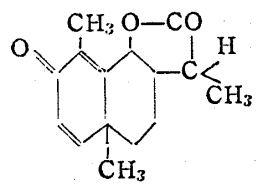
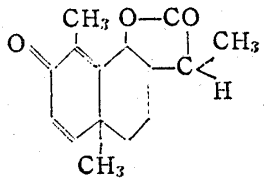


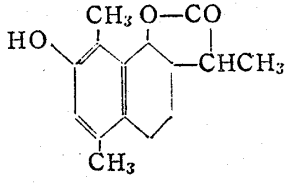
Fig. 6 *l*-α-Desmotroposantonin (in Nujol)



l-Santonin



β-Santonin



l-α-Desmotroposantonin

The authors express their thanks to Prof. Emeritus Y. Asahina and Prof. T. Momose for their encouragements throughout this study, and to Prof. G.R. Clemo for his help in sending the sample of β-santonin. Thanks are also expressed to Dr. Bando for his help in biological experiment, and to Mr. H. Kamata, Lecturer of Applied Chemistry, Mr. Tanaka, and Mr. Nishino for their kind cooperations in measurement of infrared spectra. Analyses were made by Miss Ota and Miss Kondo of the Institute of Infectious Diseases, to whom the authors' thanks are due.

Experimental

One g. of santonin was administered to a dog per day. The total amount of santonin taken reached 44 g. Sixteen L. of the urine collected was acidified with H_2SO_4, saturated with $(NH_4)_2SO_4$, and then extracted with benzene. On concentration of the extract α-hydroxysantonin separated out. The crude crystals were washed with bicarbonate and recrystallized from ethanol. Yield, 0.82 g. Colorles prisms, m.p. 286°, $[\alpha]_D^{20}$: -133.9° (c=0.6, ethanol). It gives a slight yellow coloration with methanolic solution of sodium methoxide. The rate of acetylation was measured to determine the property of hydroxyl[8]. Six mg. of α-hydroxysantonin and phenylacetic acid were heated at 155~156° for 1 hr. in a sealed tube. The rate of acetylation is 4.7% (tertiary).

Diacetate of desmotropo-α-hydroxysantonin—0.1 g. of α-hydroxysantonin was warmed on a bath with 2 cc. of acetic anhydride in the presence of 1 drop of conc. H_2SO_4. The reaction product was recrystallized from ethanol giving colorless plates, m.p. 188°, $[\alpha]_D^{18}$: -157° (c=2.0, ethanol). Yield, 95% of the theoretical amount. *Anal.* Calcd. for $C_{19}H_{22}O_6$: C, 65.89; H, 6.35. Found: C, 65.79, 65.62; H, 6.20, 6.70.

Desmotropo-α-hydroxysantonin—By hydrolysis of acetate with 0.1*N* NaOH. Coloress plates, m.p. 230~231° (from ethanol), $[\alpha]_D^{17}$: -49° (c=0.2, ethanol). *Anal.* Calcd. for $C_{15}H_{18}O_4$: C, 68.70; H, 6.78. Found: C, 68.90; H, 6.44.

Desmotropoartemisin[9]—Prepared from artemisin by the acetylation method. Diacetate, m.p. 150°, was hydrolyzed to give colorless plates, m.p. 238°.

Determination of vicinal hydroxyls by periodate reaction—0.02 g. of the respective samples of α-hydroxysantonin and artemisin were accurately weighed. Each was dissolved in a small amount of ethanol, and 1 cc. of 0.1*N* Na_2CO_3 was added. By warming on a bath for 30 mins., it was converted into sodium salt of α-hydroxysantoninic acid and artemisinic acid, respectively. The solution was cautiously neutralized with dil. acetic acid and then 1 cc. of 0.25 mol. sodium periodate solution was added. The total amount was adjusted to 5 cc. with water and the mixture was kept standing for 2 hours at a room temperature.

To 2 cc. of the reaction mixture, 5 cc. of water, 5 cc. of boric acid-borax buffer solution (3 g. of boric acid and 4 g. of borax in 100 cc. of water), and 0.1 g. of potassium iodide were added. Iodine separated was titrated with 0.1*N* As_2O_3 solution.

Amount of 0.1*N* As_2O_3 consumed corresponding to the amount of periodate recovered: 3.07 cc. for α-hydroxysantonin; 2.43 cc. for artemisin. Calculated amount of oxygen (1 mole) needed for oxidation of artemisin: 1.20 mg. Found: $(3.07-2.43)\times 5/2\times 0.80$ mg. = 1.28 mg.

A controlled test using 0.01 g. of α-hydroxysantonin was carried out under the same procedure as described above.

Amount of 0.1*N* As_2O_3 consumed correspoding to the amount of periodate recovered: 4.26 cc. for α-hydroxysantonin; 4.18 cc. for blind test. Calculated amount of oxygen (1 mole) needed, if α-hydroxysatnonin is tentatively oxidized: 0.61 mg. Found: $(4.26-4.18)\times 5/2\times 0.8$ mg. = 0.15 mg. This result would indicate that α-hydroxysantonin does not suffer periodate oxidation.

The ultroviolet absorption spectra were measured by Beckman DU Spectrophotometer, and the infrared absorption spectra by Baird Recording Photometer using Nujol mull.

Summary

The structure of α-hydroxysantonin, a metabolic product of santonin obtained from the urine of dog, was discussed. From the result of periodate oxidation and the consideration on the configuration of the desmotropo compound, it was concluded that α-hydroxysantonin is represented as 11-hydroxysantonin.

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8) Bull. Inst. Phys. Chem. Research (Tokyo), 15, 1197 (1936).
9) Bertelo: Gazz. chim. ital., 50, i, 118 (1920).