rivative identified as the di-*p*-nitrobenzoate, m.p. and mixed m.p. 187–189°.[11] The furanoside was hydrolyzed, deionized with Duolite A-4 and evaporated to yield a sirup which crystallized on seeding. The 2,3,6-tri-*O*-methyl-D-glucose had m.p. and mixed m.p. 117–120°.[14] From the initial and final rotations of the furanoside mixture it may be calculated that the glucose component amounts to 10-20% of the trimethyl sugars.

Component 5 was tentatively identified by chromatography as 2,3-di-*O*-methyl-D-mannose.

VANCOUVER, B. C., CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Oxidation of Alginic Acid with Hypochlorite at Different Hydrogen Ion Concentrations[1]

BY ROY L. WHISTLER AND RICHARD SCHWEIGER

RECEIVED JUNE 2, 1958

Alginic acid is oxidized more slowly by hypochlorite at *p*H levels of 3, 5, 7, 9 and 11 than is corn starch amylopectin. However, in correspondence with the amylopectin, oxidation proceeds most swiftly at *p*H 7 and extensive attack occurs at carbon atoms C_2 and C_3 as evidenced by the isolation of oxalic, L-tartaric and D,L-tartaric acids from the oxidized and hydrolyzed products.

Commercial polysaccharides frequently are subjected to the action of oxidizing agents either for the purpose of bleaching or for modification of properties. In previous publications[2] this Laboratory has described the action of hypochlorite on starch and on methyl 4-*O*-methyl-α,D-glucopyranoside and methyl 2-*O*-methyl-α-D-glucopyranoside.

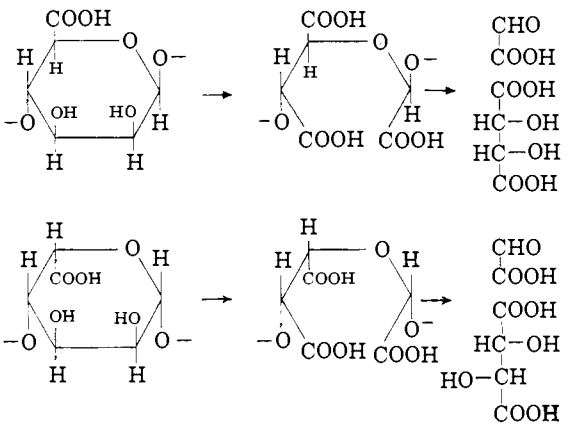
To obtain information on how changes in the grouping at carbon atom C_6 can affect the oxidation, attention is now directed at alginic acid. This polysaccharide is produced commercially in the United States from the seaweed *Macrocystis pyrifera*. It is a linear glycuronoglycan which contains[3] roughly 70% D-mannuronic acid units, presumably linked β(1 → 4), and about 30% L-guluronic acid units in, as yet unestablished but presumed, 1→4-linkages. The order of occurrence of the two types of uronic acid units is not known. However, because each sugar unit contains a carboxyl group at the C_6-position it is of interest to determine how this grouping affects the attack on the sugar units by hypochlorite.

On treatment with hypochlorite at different *p*H levels the polysaccharide is oxidized as shown in Fig. 1. It is observed, at once, that at all *p*H levels hypochlorite oxidation of alginic acid proceeds in a manner similar to the hypochlorite oxidation of amylopectin. Significantly, oxidation proceeds most swiftly at *p*H 7. However, major differences are also apparent. First, it is noted that at all *p*H levels oxidation proceeds more slowly than with corn amylopectin. Furthermore, the amount of carbon dioxide produced during the oxidation (Table I) is higher for alginic acid at all *p*H levels than for amylopectin.

TABLE I

*p*H	3	5	7	9
Moles of carbon dioxide per mole uronic acid unit per 3 moles of hypochlorite consumed	1.39	1.16	0.72	1.27

Correspondingly, the oxidative attack to cleave carbon atoms C_2 and C_3 should be less if it proceeds according to the same mechanism as postulated for amylopectin. If oxidative attack occurs in this fashion the products should be glyoxylic and L-tartaric acids from the L-guluronic acid units and glyoxylic and *meso*-tartaric acids from the D-mannuronic acid units.



Evidence for these products is obtained by isolations from the hydrolyzate of hypochlorite-oxidized algin. Presence of glyoxylic acid is indicated by isolation of oxalic acid after further oxidation of the hydrolysis products with hypoiodite (Table II). L-Tartaric acid can be isolated directly from the hydrolyzate, but *meso*-tartaric acid undergoes rearrangement[4–6] under the conditions of hydrolysis and is converted to the D,L-tartaric acid, which is isolated (Table II).

TABLE II

*p*H	Mole tartaric acid per mole uronic acid unit	Mole oxalic acid per mole uronic acid unit
3	0.032	0.035
5	.034	.047
7	.054	.068
9	.060	.081

The yield of oxalic and tartaric acids suggests that only about 6–7% of the hypochlorite not

(1) Journal Paper No. 1293 of the Purdue Agricultural Experiment Station, Purdue University, Lafayette, Ind.

(2) (a) R. L. Whistler and S. J. Kazeniac, *J. Org. Chem.*, **21**, 468 (1956); (b) R. L. Whistler, E. S. Linke and S. J. Kazeniac, THIS JOURNAL, **78**, 4704 (1956); (c) R. L. Whistler and R. Schweiger, *ibid.*, **79**, 6460 (1957).

(3) R. L. Whistler and K. W. Kirby, unpublished results.

(4) M. A. F. Holleman, *Rec. trav. chim.*, **17**, 77 (1896).

(5) M. Jungfleisch, *Bull. soc. chim.*, **19**, 100 (1873).

(6) Chr. Winther, *Z. physik. Chem.*, **56**, 508 (1906).

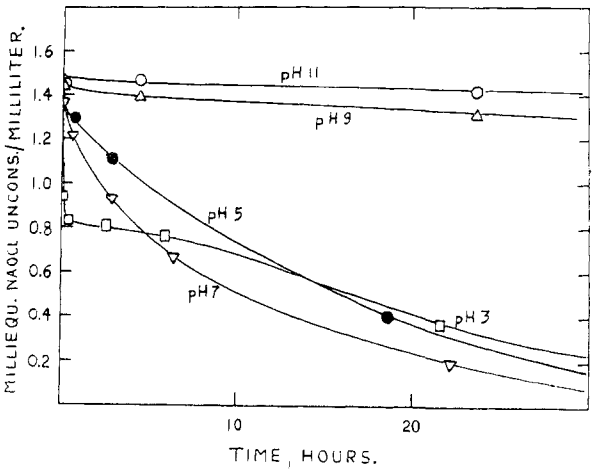


Fig. 1.—Hypochlorite consumption by algin at 25°.

converted to chlorate is consumed in the cleavage of carbon atoms C_2 and C_3. This is considerably lower than in the similar oxidation with corn amylopectin where about 25% of the hypochlorite was consumed in this specific cleavage. Since the yields of glyoxylic and tartaric acids appear largest at *p*H 7 or higher, the enediol mechanism previously suggested for the oxidation of amylopectin seems to be applicable. The lower yields of cleavage products compared to the yields from amylopectin are not fully explained. However, it is of interest to note that at *p*H 7 the yields are raised from about 6% to 11–13% when the hypochlorite concentration is increased to 6 moles per mole of uronic acid unit.

TABLE III

*p*H	Mole uronic acid per mole initial uronic acid unit
3	0.350
5	.429
7	.423
9	.340

With 3 moles of hypochlorite per mole of uronic acid unit, not all of the uronic acid units are oxidized, as evidenced by the finding of both D-mannuronic and L-guluronic acids in the products from the oxidized and hydrolyzed algin. Amounts of these unoxidized uronic acids in the hydrolyzate are shown in Table III. These values are determined by measurement of the carbon dioxide evolved on boiling with 12% hydrochloric acid.

Experimental

Chromatography.—Chromatographic analyses were made using chromatographic grade Whatman No. 1 filter paper.

Irrigant A was a volumetric mixture of ethyl acetate–acetic acid–formic acid–water (18:3:1:4) and irrigant B, a mixture of ethyl acetate–pyridine–water (10:4:3). Spray reagents used were: I, 1% potassium permanganate in 2% sodium carbonate solution mixed with 2% sodium periodate solution[7] in a ratio of 1:4; II, a methanolic solution of hydroxylamine, followed by a 2% solution of ferric chloride in 1% hydrochloric acid[8]; III, a mixture of 2% silver nitrate and 5 *N* ammonium hydroxide in a ratio of 1:1[9]; IV, aniline hydrogen phthalate in butanol–ethanol–water.[10]

(7) R. U. Lemieux and H. F. Bauer, *Anal. Chem.*, **20**, 920 (1954).
(8) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 5859 (1951).
(9) S. M. Partridge, *Nature*, **158**, 270 (1946).
(10) S. M. Partridge, *ibid.*, **164**, 443 (1949).

All flow rates (R_g) are compared to that of D-glucose.

Rate of Oxidation.—Two and one-half grams of sodium alginate was dissolved in 250 ml. of water by stirring for several hr. The solution was then divided into 5 equal parts which were adjusted to *p*H 3, 5, 7, 9 and 11 by addition of dilute hydrochloric acid or sodium carbonate. To avoid precipitation of alginic acid, dilute hydrochloric acid was added carefully with stirring. To each sample was added 50 ml. of *N* sodium hypochlorite solution (3 moles of hypochlorite per mole of sugar unit) adjusted to the same *p*H as the corresponding algin solution. Samples were maintained in the dark at 25°. Hydrogen ion concentration was frequently determined and corrected when necessary.

Hypochlorite consumption was followed by sodium thiosulfate titration of acidified aliquot portions containing potassium iodide. Actual amounts of hypochlorite consumption are shown in Fig. 1. The percentages of hypochlorite transformed at different *p*H values to chlorate during the total time of each oxidation are

*p*H	3	5	7	9
Hypochlorite to chlorate, %	6.04	8.85	52.8	7.71

Chlorate determinations were made on 1-ml. aliquots to which were added crystalline potassium iodide and 4–5 ml. of concentrated hydrochloric acid. After diluting with water to about 10 volumes, free iodine was titrated with 0.1 *N* sodium thiosulfate solution.

After all the hypochlorite was consumed the chlorate concentration remained constant, over the several days it was measured, showing that it is not an oxidant for alginate under these conditions.

Hydrolysis of Oxidized Algin.—To samples from complete oxidation at *p*H 3, 5 and 7 were added 3 ml. of concentrated sulfuric acid to make the solution about 1 *N* in sulfuric acid. Sulfur dioxide was introduced for about 15 min. to reduce the chlorate, the solution was refluxed for 5 hr., and sulfate ion precipitated quantitatively as barium sulfate from the hot solution. Hot barium hydroxide solution was added until the acidity was reduced to a very low level and then the precipitation was completed with barium chloride. After cooling, barium sulfate was filtered and the clear, dark brown solution passed through a column of cation exchange resin IR-120 (H). The solution was concentrated under reduced pressure to a brown sirup and examined chromatographically. Chromatograms at all three *p*H levels showed a long brown streak containing several spots which were the same for all *p*H levels although the intensity of components differed somewhat.

Chromatographic Examination.—To obtain larger quantities of products for identification, 10 g. of sodium alginate were oxidized with 6 moles of sodium hypochlorite per mole of uronic acid unit at *p*H 7 in the same manner as before, except that the oxidant was added in two portions, the first portion being exhausted before the next was added. A large amount of hypochlorite was added because of its high conversion to chlorate at *p*H 7. At the end of the reaction, chlorate was reduced with sulfur dioxide and the polymer hydrolyzed by sulfuric acid and ion-exchanged as described above. The brown effluent did not decolorize with charcoal. It gave the same chromatographic components as the previous smaller lots. The effluent was continuously extracted with ethyl ether for 5 days. Both the ether extract and the aqueous solution were concentrated to brownish yellow sirups. These were chromatographed on paper with irrigant A. The chromatograms again showed a long streak, but the spots were clearer.

Two strong spots from the chromatogram of the ether-soluble mixture had R_g values identical with those of known tartaric and glyoxylic acids. Five other very weak spots were observed which move fast with both acid and basic irrigants A and B. The second component, R_g 5.61 in irrigant A, reacted instantly with spray reagent III. No additional components were indicated by use of spray reagents I or IV.

Chromatography of the aqueous residue showed three main components with flow rates identical to L-gulurono-lactone, D-mannuronolactone and their mixed free acids.

D,L-Tartaric Acid.—The cation-exchanged sirup described earlier was diluted, calcium acetate was added in excess and the *p*H was adjusted to 3 by addition of sodium hydroxide solution. After 2 days the precipitate was filtered and washed with water. Calcium salts were suspended in water and stirred with cation exchange resin IR-

120 (H) for several hr. The filtrate was treated with charcoal and concentrated to a small volume and put into a vacuum desiccator over calcium chloride. The separated crystals showed a m.p. of 205–206°, undepressed when mixed with authentic D,L-tartaric acid.

Unknown Component.—A smaller amount of calcium salt precipitated when the aqueous solution above was being neutralized. Since the colorless product did not crystallize after conversion to the free acid by cation exchange resin, the dry sirup was dissolved in methanol and treated with diazomethane in ether. The ester was concentrated to a sirup, taken up in anhydrous methanol saturated with ammonia and allowed to stand at 0° for several hr. After concentration to a sirup and dissolution in hot ethanol, crystals formed on cooling which were freed from sirup on a porous plate and recrystallized from a mixture of ethanol and ethyl acetate. After a second recrystallization the thin prismatic plates melted at 134–135°. The compound is optically inactive.

The infrared spectrum showed strong absorption bands at wave lengths of 3.18, 7.13, 7.83, 7.96, 8.20, 9.34, 9.70, 12.92 and 13.06 μ, and probably two weaker bands at 8.40 and 8.60 μ. This analysis indicated that no carbonyl group is present. The Beilstein test showed the presence of sulfur, but not of chlorine. This test, which produced hydrogen sulfide after acidifying, proved that sulfur is not present as sulfonic acid nor as one of its derivatives. According to the analysis, the empirical formula is $[C_1H_7O_{4.5}N_1S_1]_x$, if the remaining percentage is considered as oxygen. The values for hydrogen and oxygen are not as accurate as the others.

Anal. Found: C, 9.43; H, 5.28; N, 11.00; S, 23.70.

Components of the Ether Extract.—Ether extract from the large preparation was concentrated to a sirup and placed on a cellulose column, 45 × 600 mm., and irrigant A applied. The first effluents on concentration produced crystals in less than 1% yield which were removed by filtration, washed with ethyl acetate and recrystallized from a mixture of ethanol and ethyl acetate. They had no sharp melting point, but sintered between 220–225° with decomposition in the range 240–250°. They were optically inactive. Chromatographed with irrigant A, the crystals gave two components with R_g values of 1.89 and 2.66. Both reacted instantly with spray reagent I, indicating the presence of glycols, and both reacted with spray reagent II, indicating a lactone. When the crystalline material was chromatographed with irrigant B only one component, R_g 0.831, was present on the chromatogram.

Part of this material was dissolved in methanol and methylated with diazomethane in ether. On evaporation of the solvent, crystals appeared which were recrystallized from a mixture of methanol and ethyl ether. The long, flat prisms were filtered, washed with ether and dried. When heated rapidly, the crystals melted at 198°; if heated slowly, they decomposed before reaching the melting point.

In an attempt to form an amide, another part of the material was methylated with diazomethane and then treated with ammonia in methanol as previously described. After evaporation of the solvent under reduced pressure the crystalline residue was dissolved in 95% ethanol. During evaporation of the solvent in a desiccator, crystals appeared as long, thin prisms. They did not melt, but decomposed between 260–270°.

The small amount of sirup separated from the first crop of crystals with R_g 1.89 was chromatographed again on a cellulose column, 25 × 600 mm., but with irrigant B. The principal component on paper had an R_g of 4.59 with irrigant B. It remained as a brown sirup. This sirup was dissolved in acetic anhydride and a trace of perchloric acid was added as a catalyst. After standing for several hr. at room temperature, the mixture was poured into water and then extracted with ether. The ether layer was dried with sodium sulfate, and, after evaporation, a brown sirup remained which was distilled under reduced pressure. The acetylated product distilled as a thin colorless sirup, $[\alpha]^{25}D$ +6.9 ± 1.0° (*c* 1.7 in methanol), at a pressure of 0.05 mm. and a bath temperature of 100–120°. After the sirup was kept at −15° for several days it crystallized. It was then dissolved in methanol, and water was added until the solution became turbid. On seeding and standing at −15° the mixture formed crystals as long prisms which were filtered, washed with a mixture of methanol and water and dried, m.p. 62–64°, $[\alpha]^{25}D$ + 5.2 ± 1.2° (*c* 1.0 in methanol). After a second recrystallization the substance melted sharply at 66.5°. Rast molecular weight[11] was 270 ± 5%.

The infrared spectrum showed strong absorption bands at wave lengths of 5.73, 7.25, 7.80, 8.25, 8.81 and 10.28 μ, and weaker bands at 3.36, 6.93, 9.75, 10.71, 11.51, 12.87, 13.57 and 15.38 μ. This analysis gave clear evidence of acetyl groups. According to the analysis the empirical formula is $[C_2H_3O_{1.3}]_x$, if the remaining percentage is considered as oxygen.

Anal. Found: C, 50.04; H, 6.35; $COCH_3$, 9.00.

The major individual component from the cellulose column crystallized when the effluent portion containing it was concentrated. Crystals were washed with ethyl acetate and recrystallized from ethanol–ethyl acetate mixture; m.p. 168.5–170°, undepressed when admixed with L-tartaric acid, $[\alpha]^{25}D$ +15.2 ± 0.6° (*c* 2.0 in water). The acid was esterified to the dimethyl derivative and this was converted to the diamide by the same methods used above for the preparation of L-tartaramide; m.p. 197–199° dec., $[\alpha]^{25}D$ +107.4 ± 3.0° (*c* 0.7 in water).

A separate portion of concentrated ether extract, which showed no chromatographic evidence of oxalic acid, was treated with hypoiodite solution at *p*H 10 to oxidize glyoxylic acid to oxalic acid. After acidification, excess iodine was reduced with an equivalent amount of thiosulfate. Oxalic acid was precipitated from the solution at *p*H 6 with calcium acetate. The precipitate was dissolved in dilute acid and reprecipitated by addition of ammonium hydroxide. The precipitate was stirred with water and Amberlite IR-120 (H) for several hr., filtered, concentrated and cooled to produce crystals of oxalic acid, m.p. 187–189°, undepressed by admixture with authentic sample.

L-Guluronolactone.—The ether-extracted water solution was separated on a cellulose column, 45 × 600 mm., with irrigant A. First effluent fractions contained, in addition to L-guluronolactone, two components which moved rapidly on paper, R_g 5.48 and 4.47, and gave slight brown spots with spray reagent IV. These components were not present in the next set of fractions which contained only L-guluronolactone. The fractions were concentrated, the sirup taken up in water, decolorized with charcoal and again concentrated. It was dissolved in 25% nitric acid solution and heated at 50–60° for 1 hr. The solution was concentrated at reduced pressure, dissolved in 0.5 ml. of 50% potassium hydroxide solution and the *p*H adjusted to 6 by addition of glacial acetic acid. On standing at 2° for several days, crystals of the monopotassium salt of D-glucaric acid separated and were washed with ice-water; m.p. 182–185° dec., on recrystallization from water, m.p. 188–191° dec., unchanged by admixture with authentic sample; $[\alpha]^{25}D$ +5.3 ± 1.0° (*c* 0.5 in water).

D-Mannuronolactone.—The next fraction from the cellulose column likewise was concentrated, decolorized and oxidized with nitric acid. This product was also similarly methylated with diazomethane and converted to the diamide with ammonia in methanol. During concentration, the solution deposited crystals which were recrystallized from aqueous ethanol; m.p. 187–189° dec., unchanged by admixture with authentic mannaramide, $[\alpha]^{25}D$ −22.5 ± 2.0° (*c* 1.2 in water).

Determination of Amounts of Organic Acids Found at Different *p*H Values.—The yield measurements of the various organic acids formed at 25°, oxidations were conducted at *p*H levels of 3, 5, 7 and 9 on 4-g. portions of sodium alginate dispersed in 450 ml. of buffered solution with 3 moles of hypochlorite per mole of uronic acid unit. After the oxidant was consumed, chlorate was reduced with sulfur dioxide, the polymer was hydrolyzed with 1 *N* sulfuric acid, sulfate ions were removed as barium sulfate, cations were removed by exchange resins and volumes were adjusted to 50 ml.

Glyoxylic acid was measured by conversion to oxalic acid. Ten-ml. aliquot portions were oxidized with hypoiodite[12] and the oxalic acid present precipitated as the calcium salt and, after washing, determined by titration with permanganate.

Yields of tartaric acid were obtained from the weights of the calcium salts. For this, calcium acetate was added to 10-ml. aliquot portions, the *p*H was adjusted to 6, and the solutions were concentrated to 6–8 ml., whereupon calcium

(11) K. Rast, *Ber.*, **55**, 1051, 3727 (1922).

(12) R. Willstätter and G. Schudel, *ibid.*, **51**, 780 (1918).

tartrate crystallized over 3 days. The precipitates were washed, dried and weighed for estimation of tartrate present.

Uronic acid present was determined in 15-ml. aliquot portions by measurement of the carbon dioxide evolved when the solutions were made 12% in hydrochloric acid and heated.[13,14] Yields of glyoxylic and tartaric acids are shown in Table II and the yields of uronic acids are shown in Table III.

Carbon Dioxide Evolved.—Four grams of sodium alginate was dissolved in each of four 300-ml. portions of water and the solutions adjusted one each to *p*H 3, 5, 7 and 9. Adjustment to *p*H 3 was obtained by addition of hydrochloric acid to lower the *p*H value, which then was buffered at 3 by addition of monopotassium phosphate. Adjustment to *p*H 5 and 7 was obtained by addition of small quantities of mono- and dipotassium phosphate in appropriate ratios. Adjustment to *p*H 9 was obtained with dipotassium phosphate. To each solution in a closed vessel hypochlorite solution was added to the level of 3 moles per mole of sugar unit and the volumes adjusted to 450 ml. each. Evolution of carbon dioxide was measured as described in an earlier report.[2c] Results are shown in Table I.

LAFAYETTE, IND.

(13) K. U. Lefèvre and B. Tollens, *Ber.*, **40**, 4513 (1907).

(14) R. L. Whistler, A. R. Martin and M. Harris, *J. Research Natl. Bur. Standards*, **24**, 13 (1940).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, TAKEDA PHARMACEUTICAL INDUSTRIES, LTD., AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

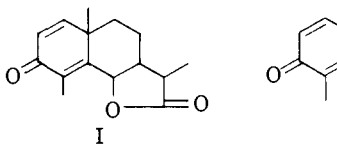
The Stereochemical Correlation of (−)-α-Santonin, Artemisin and ψ-Santonin

BY M. SUMI, WILLIAM G. DAUBEN AND WILLIAM K. HAYES

RECEIVED JUNE 9, 1958

Artemisin, the 7-hydroxy derivative of (−)-α-santonin, has been degraded to α-(1,4-dimethyl-7-hydroxy-5,6,7,8-tetrahydro-6-naphthyl)-propionic acid lactone (IV), a compound also derivable from ψ-santonin. This result establishes a common stereochemistry for C_6, C_7 and C_{11} in these two series of sesquiterpenic acid lactones. Other workers previously have established a similar relationship for the C_9-angular methyl group.

From a variety of species of Artemisia, the sesquiterpenic acid lactones (−)-α-santonin (I), artemisin (II) and ψ-santonin (III) have been obtained.[1] These compounds all possess a common



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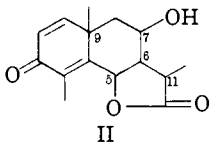
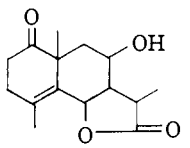
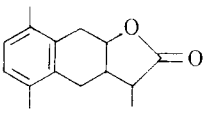


II



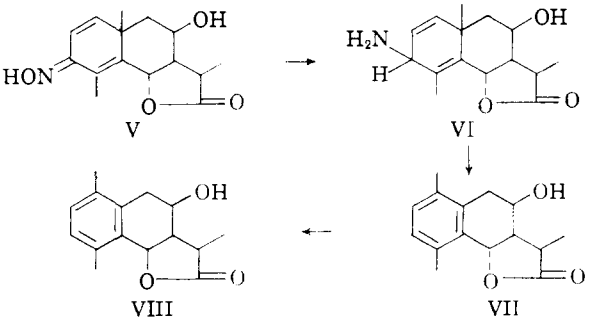
III

carbon skeleton but differ as to the number and placement of certain functional groupings. In view of the several common structural characteristics as well as common biological origins of these compounds, it was of interest to relate the absolute configurations of the various asymmetric centers present in them. It has been shown[2] recently that artemisin (II) is a 7-hydroxy derivative of (−)-α-santonin. Since II possesses characteristics of santonin in ring A and characteristics of ψ-santonin in ring B, it can serve as a relay in the correlation of the configurations of these two series of sesquiterpenic acid lactones. Of the five asymmetric centers in ψ-santonin (III), it already has been established, on the basis of optical rotatory dispersion measurements,[3] that the C_9-angular methyl group has the same absolute configuration as in (−)-α-santonin. The remaining centers are in ring B and by conversion of artemisin (II) to α-(1,4-dimethyl-7-hydroxy-5,6,7,8-tetrahydro-6-naphthyl)-propionic acid lactone (IV), which has been prepared from ψ-santonin,[4] the stereochemistry of C_6, C_7 and C_{11} of the two series could be related.



IV

Artemisin (II) was converted to its oxime V which upon reaction with zinc and sulfuric acid in the presence of copper sulfate was transformed into artemisinamine sulfate (VI). Due to the instability of the amine, it was not isolated but was heated directly with aqueous acid in order to transform it into hypoartemisin (VII). When the latter compound was heated in acetic acid with zinc, the conditions required to convert hyposantonin to hyposantonous acid,[1] only acetylation of the hydroxyl group occurred. Since, in general, a *cis*-lactone undergoes reductive fission more readily



V VI VIII VII

than the *trans* isomer, hypoartemisin with the *trans*-lactone was converted into its *cis* isomer, iso-

(1) J. Simonsen and D. H. R. Barton, "The Terpenes," Vol. III, University Press, Cambridge, England, 1951.

(2) M. Sumi, *Proc. Japan Acad.*, **32**, 684 (1956); **33**, 153 (1957); *Pharm. Bull.*, **5**, 187 (1957).

(3) C. Djerassi, R. Riniker and B. Riniker, THIS JOURNAL, **78**, 6362 (1956); see also, H. Bruderer, D. Arigoni and O. Jeger, *Helv. Chim. Acta*, **39**, 858 (1956).

(4) W. G. Dauben, P. D. Hance and W. K. Hayes, THIS JOURNAL, **77**, 4609 (1955).