[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

The Constitution of Sapote Gum. I. Methanolysis of Sapote Gum Methyl Ether

BY E. V. WHITE

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Methanolysis of sapote gum methyl ether furnishes a sirupy mixture of glycosidic and uronosidic components. The glycosidic components have been separated and identified as being the methyl glycosides of 3-methyl-D-xylose, 2,3,4-trimethyl-D-xylose and 2,3,4-trimethyl-L-arabinose. The uronosidic components of the methanolysis sirup have not been investigated.

The plant gums are usually described[1,2] as exudations of polysaccharide character which form in the tree or shrub either spontaneously or as a result of causative agencies. The present study deals with sapote gum[3] which forms slowly in the wounds made in the sapote tree following the collection of chicle. It is a heteropolysaccharide composed of D-xylose, L-arabinose and D-glucuronic acid units[4] but no hexose sugar has been identified in the products of aqueous acid hydrolysis.[4a] The gum is soluble in water and can be separated with some difficulty from extraneous components. It finds use as a sizing agent for cloth and felt, as a glue and has been tested as a beater additive in the manufacture of paper. The methyl derivative is soluble in water and in the lower molecular weight alchols but is insoluble in other solvents, possibly because of a relatively low methoxyl content and a high percentage of uronic acid groups. The ether derivative forms remarkably tough, colorless, transparent films. In view of this, side-chain branching from the main chain of the macromolecule may be limited in extent.

In order to study the structural character of the polysaccharide the methyl ether derivative, prepared by the Haworth method, was subjected to methanolysis. The resulting sirup was separated into the respective glycosidic and uronosidic fractions and the components of the former fraction were identified. Since the gum shows considerable resistance to complete methanolysis, it is to be expected that some portion of the biuronosidic components are resident in the acid fraction. This fraction has not been examined in the course of the present investigation, so that quantitative data as to the molecular ratio of the different monose units cannot be given at the present time.

The glycosidic fraction was separated readily by distillation into two main fractions. One of these was shown to be methyl 3-methyl-D-xyloside by conversion into the known crystalline sugar and preparation of the crystalline anilide. In the original molecule, therefore, certain of the xylose units must serve as branch points for other units at the 1-, 2- and 4-positions, unless the unlikely furanoid structure prevails when the 5- rather than the 4-position would be engaged. The second fraction of the glycosidic sirup consisted of fully alkylated pentoside units which obviously are derived from the terminal units of the macromolecule. When the hydrolyzate of this fraction was examined chromatographically, two components were indicated; these were separated on a column of acid-washed Magnesol. One of the components, the faster moving of the two compounds under the action of the developer, proved to be 2,3,4-trimethyl-D-xylose, identified both as the crystalline sugar and as the corresponding lactone. The slower moving component was shown to be 2,3,4-trimethyl-L-arabinose. This compound was identified by conversion into the corresponding lactone and preparation of the crystalline amide and phenylhydrazide. The latter showed no depression in melting point when mixed with an authentic specimen. As a final proof of structure, when the sugar was oxidized with nitric acid, 2,3,4-trimethyl-L-araboglutaric acid was formed in good yield. The latter compound was identified through the ester as the corresponding diamide.

It would thus appear that the molecular architecture of sapote gum must provide for terminal residues of L-arabopyranose and D-xylopyranose. A portion of these, if not all, must be united glycosidically with units of xylose at the 2- or 4-positions of the latter. Depending upon the positions occupied by the terminal residues, the disubstituted units of D-xylose may be glycosidically united with each other through the alternate positions. The extent of the present evidence does not justify the conclusion that the sapote molecule comprises in large part a single chain of D-xylose units, each of which bears a substituent of D-xylopyranose or L-arabopyranose of terminal character. It would appear, however, that the polysaccharide contains no primary hydroxyl groupings.

In view of the unusual nature of the arabinose unit isolated from the glycosidic products of methanolysis, the possibility that some unforeseen alteration of ring structure had occurred was given careful consideration. No evidence was found to indicate that free arabinose was present as an extraneous component of the gum or that the sugar had been hydrolyzed from the polysaccharide and entrained therewith throughout the course of the preparatory reactions preceding methanolysis. The uronosidic portions of the gum are now being examined.

Experimental Part

Methylation of Sapote Gum.—Fifty grams of sapote gum was dissolved in 200 ml. of water and placed in a three-liter, three-necked flask equipped with an efficient sweep-stirring device, a condenser and two graduated dropping funnels. Dimethyl sulfate (175 ml.) and 350 ml. of 30% sodium hydroxide were added dropwise and simultaneously over a

(1) A. G. Norman, "The Biochemistry of Cellulose, the Polyuronides, Lignin, Etc.," Clarendon Press, Oxford, 1937, p. 121.

(2) J. K. N. Jones and F. Smith, *Advances in Carbohydrate Chem.*, **4**, 243 (1949).

(3) The gum is probably taken from *Sapotaceae achras* and can be obtained from the Asher Kates Company, Lima, Peru.

(4) E. Anderson and H. D. Ledhetter, *J. Am. Pharm. Assoc.*, **40**, 623 (1951).

(4a) G. G. S. Dutton and G. Kilgour (private communication) report the presence of D-galactose in the hydrolyzate of some samples of sapote gum.

period of 3 hours. Acetone was added to the reaction when necessary to facilitate stirring. The temperature was maintained at 25° throughout the reaction by means of a water-bath. After addition of the reagents, the reaction mixture was heated to 50° for 1 hour, cooled and dialyzed against running water for 2 days to remove inorganic salts. During this period extraneous material, such as bark, dirt, chips, etc., and a small gelatinous precipitate[4] settled out, leaving a clear solution of partially methylated sapote gum. The dialyzate was concentrated to a sirup under reduced pressure and remethylated under similar conditions. After four treatments methylation was complete. The dialyzed solution of the sodium salt of sapote gum methyl ether was separated from sodium ion on a column of Amberlite resin IRC-50, and the free acid evaporated to dryness. The product was soluble in water and in methyl and ethyl alcohol but was insoluble in other common solvents: yield 33 g. (Found: OMe, 36.0; uronic acid, 26.7.)

In a separate exploratory experiment, an aqueous solution of the crude gum was examined chromatographically for the presence of arabinose. A similar solution containing 1% of arabinose was also examined after dialysis for 24 hours against running water. No evidence of the presence of arabinose was found in either instance.

Methanolysis of the Free Acid of Methylated Sapote Gum.—Thirty-two grams of the free acid of sapote gum methyl ether was dissolved in 200 ml. of absolute methyl alcohol and anhydrous hydrogen chloride added to 3.5% concentration. The reaction mixture was heated under reflux for 8 hours, cooled, neutralized with silver carbonate, filtered and evaporated to a sirup. This was extracted with petroleum ether and the residual sirup subjected to remethanolysis with 3% methanolic hydrogen chloride in a sealed tube at 110° for 5 hours. Residual acidity was removed with silver carbonate and the filtered solution, freed from solvent, was combined with the petroleum ether extract to yield a mixture of the glycosides and uronosidic components; yield 32.5 g.

Separation of Uronosidic and Glycosidic Components.—The above sirup (32.5 g.) was dissolved in 0.3 *N* barium hydroxide and warmed at 60° for 2 hours in order to saponify the uronoside esters and to form the corresponding barium salts. Excess barium hydroxide was neutralized with carbon dioxide and the precipitated barium carbonate was removed by filtration. Water was removed by vacuum distillation and by co-distillation with neutral anhydrous chloroform. Trituration of the sirup with anhydrous ether then extracted the soluble glycosidic components (17.0 g.), leaving the barium uronosides as an insoluble residue; yield 24.0 g. The latter fraction has not been examined.

The glycosidic portion was distilled fractionally under high vacuum to furnish fraction I, 6.8 g., b.p. 63° at 0.2 mm.; fraction II, 6.0 g., b.p. 65–85° at 0.2 mm.; fraction III, 4.2 g., b.p. 92° at 0.2 mm.

Isolation and Identification of 3-Methyl-D-xylose.—A portion of fraction III (3.0 g.) was heated with 60 ml. of 0.575 *N* sulfuric acid on a boiling water-bath at 92° for 6 hours when the rotation of the solution reached a constant value. Sulfuric acid was removed as sulfate with barium carbonate and the filtered solution was concentrated to a sirup, dried by distillation with anhydrous methanol and extracted with acetone. Upon concentration to a thin sirup the acetone solution deposited crystals; yield 2.0 g. Recrystallization from acetone furnished 3-methyl-D-xylose,[5] m.p. 104°, $[\alpha]_D$ 15° (*c* 1.16, equilibrium in water at 20°).

Anal. Calcd. for $C_6H_{12}O_5$: OMe, 18.9. Found: OMe, 18.9.

One-half gram of 3-methyl-D-xylose in 10 ml. of absolute ethanol was heated under reflux with 0.3 g. of redistilled aniline for 2 hours. The solvent was then removed under reduced pressure and the residual sirup crystallized completely from acetone to furnish the anilide of 3-methyl-D-xylose,[6] m.p. 137°, $[\alpha]_D$ 80° (*c* 1.5, ethyl acetate at 20°).

Anal. Calcd. for $C_{12}H_{15}O_4N$: OMe, 13.9; N, 6.3. Found: OMe, 13.8; N, 5.7.

Isolation and Identification of 2,3,4-Trimethyl-D-xylose.—An exploratory investigation of the hydrolyzate of fraction I by paper partition chromatography indicated the presence of two compounds and it was found that these could be separated on a column of acid-washed Magnesol[7] by the extrusion, streak and sectioning technique.

Accordingly 5.5 g. of fraction I was hydrolyzed by boiling under reflux with 70 ml. of *N* sulfuric acid for 2.5 hours when the rotation of the solution became constant. The free sugar was isolated in the usual manner and distilled under high vacuum, b.p. 100° at 0.2 mm., yield 5.03 g. The distillate was then dissolved in 100 ml. of benzene and chromatographed on a No. 6 column of Magnesol, using two liters of 50:1 petroleum ether–ethanol as developer. The column was extruded, streaked, sectioned and eluted with acetone in the usual manner. Upon removal of solvent there was thus obtained fraction I_b (2.75 g.) and fraction I_t (2.28 g.).

Fraction I_b crystallized spontaneously after distillation under high vacuum, b.p. 97° at 0.2 mm. Recrystallization from ether furnished 2,3,4-trimethyl-D-xylose,[8] m.p. 91°, $[\alpha]_D$ 18° (*c* 2, equilibrium in water at 20°).

Anal. Calcd. for $C_8H_{16}O_5$: OMe, 48.4. Found: OMe, 48.4.

Oxidation of fraction I_b (0.9 g.) with 2 ml. of bromine in 20 ml. of water for two days in the dark produced a non-reducing solution. The product of the reaction was isolated in the usual manner, distilled under high vacuum (b.p. 95° at 0.2 mm.) and crystallized from ether–petroleum ether. Recrystallization from the same solvent mixture furnished 2,3,4-trimethyl-D-xylonolactone,[9] m.p. 54°, $[\alpha]_D$ 20° (*c* 1.5, equilibrium in water at 20°).

Anal. Calcd. for $C_8H_{14}O_5$: OMe, 48.9. Found: OMe, 48.9.

Isolation and Identification of 2,3,4-Trimethyl-L-arabinose.—Fraction I_t from the chromatographic column was distilled under high vacuum (b.p. 100° at 0.2 mm.), to furnish a colorless sirup which failed to crystallize; yield 2.28 g., $[\alpha]_D$ 15° (*c* 1.7, equilibrium in water at 20°).

Anal. Calcd. for $C_8H_{16}O_5$: OMe, 48.4. Found: OMe, 48.0.

One gram of the sirup was oxidized with 2 ml. of bromine in 20 ml. of water for 2 days in the dark at room temperature when the solution became non-reducing. The reaction product was isolated in the usual manner and distilled under high vacuum (b.p. 100° at 0.2 mm.), furnishing a sirup which did not crystallize; yield 0.9 g., $[\alpha]_D$ 24° (*c* 1.2, equilibrium in water at 20°).

Anal. Calcd. for $C_8H_{14}O_5$: OMe, 48.9. Found: OMe, 49.2.

Treatment of the lactone (0.3 g.) with ammonia dissolved in anhydrous methanol for two days in the refrigerator, followed by removal of solvent, furnished a sirup which crystallized from acetone–ether solution. Recrystallization from the same solvent mixture gave the amide of 2,3,4-trimethyl-L-arabonic acid,[10] m.p. 103°.

Anal. Calcd. for $C_8H_{17}O_5N$: OMe, 45.0; N, 6.8. Found: OMe, 45.1; N, 6.6.

When the lactone (0.166 g.) was dissolved in ether and heated under reflux for 3 hours with 1.1 moles of phenylhydrazine, the hydrazide crystallized in the reaction flask. Recrystallization from alcohol–ether solution furnished the hydrazide of 2,3,4-trimethyl-L-arabonic acid,[11] m.p. 156° and not depressed upon admixture with a known specimen.[12]

The free sugar (1.77 g. of fraction I_t) was oxidized with 35 ml. of nitric acid (*d* 1.2) at 90° for 5 hours when the evolution of the oxides of nitrogen was complete. The solution was then diluted with water and distilled under reduced pressure with periodic addition of fresh water and finally of absolute methanol to remove nitric acid. The resulting sirup, in ether solution, was treated with diazomethane to destroy residual nitric acid and esterify the reaction product. Distillation of the latter under high vacuum furnished 1.83

(5) P. A. Levene and A. L. Raymond, *J. Biol Chem.*, **102**, 331 (1933).

(6) R. A. Laidlaw and E. G. V. Percival, *J. Chem. Soc.*, 1605 (1949).

(7) I. A. Pearl and E. E. Dickey, THIS JOURNAL, **73**, 863 (1951).

(8) F. P. Phelps and C. B. Purves, *ibid.*, **51**, 2443 (1929).

(9) W. N. Haworth and G. C. Westgarth, *J. Chem. Soc.*, 886 (1926).

(10) R. W. Humphreys, J. Pryde and E. T. Waters, *ibid.*, 1302 (1931).

(11) F. Smith, *ibid.*, 749 (1939).

(12) The known sample was kindly supplied by Professor Fred Smith.

g. of a mobile sirup which failed to crystallize, b.p. 100° at 0.2 mm., $[\alpha]_D$ 38° (*c* 3.79, methanol at 20°).

Anal. Calcd. for $C_{10}H_{18}O_7$: OMe, 62.0. Found: OMe, 60.5.

When 0.5 g. of the sirup was treated with methanolic ammonia in the refrigerator for two days the solution deposited crystals. Upon removal of gas and solvent, the diamide of 2,3,4-trimethyl-L-araboglutaric acid[13] was obtained, m.p. 233° (recrystallized from methanol), $[\alpha]_D$ 42.6° (*c* 3.5, water at 20°).

Anal. Calcd. for $C_8H_{16}O_5N_2$: OMe, 42.3; N, 12.7. Found: OMe, 42.2; N, 12.2.

(13) E. L. Hirst and G. J. Robertson, *J. Chem. Soc.*, **127**, 362 (1925).

Examination of Fraction II.—Fraction II proved to be a mixture of the compounds identified in fractions I and III. After hydrolysis with *N* sulfuric acid for 2 hours the free sugars were isolated in the usual manner and examined by paper partition chromatography using the previously identified compounds as reference standards. There was no indication of the presence of any substance other than those characterized.

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APPLETON, WISCONSIN

[CONTRIBUTION FROM RIKER LABORATORIES, INC.]

The Structure of Isorubijervine. Conversion to Solanidane and Solanidane-3β-ol[1]

BY FRANK L. WEISENBORN AND DIANA BURN

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Isorubijervine has been established as a hydroxy derivative of solanidine by conversion to solanidane and solanidane-3β-ol. Treatment of isorubijervine with *p*-toluenesulfonyl chloride led to a unique, salt-like tosyl derivative showing that the primary hydroxyl must be within bonding range of the nitrogen. Carbon 19 was thus eliminated as a site for this hydroxyl.

Only one of the veratrum alkaloids, rubijervine, has been shown to have a steroidal type ring system and to be related to solanidine. This was demonstrated by Sato and Jacobs,[2] who converted rubijervine to solanidine and solanidane-3β-ol.

Isorubijervine, another alkaloid from *Veratrum viride* was also studied by Jacobs and co-workers.[3-6] This substance was found to contain two hydroxyl groups, one of which was secondary and at carbon 3. The other was shown to be of primary character by the oxidation of dihydroisorubijervine to an acid which could be reconverted to dihydroisorubijervine by reduction of the corresponding ester with lithium aluminum hydride. Since the ester was difficult to saponify and since selenium dehydrogenation of isorubijervine yielded 1,2-cyclopentenophenanthrene, isorubijervine was assigned a hydroxysolanidine structure (I) with the primary hydroxyl group at carbon 18. No evidence for a solanidine type ring system was obtained, however. We have now shown that isorubijervine is in fact a derivative of solanidine by conversion to solanidane-3β-ol and solanidane.

Oppenauer oxidation of dihydroisorubijervine (II) yielded solanidane-3-one-18-ol (III) which was then further oxidized to the solanidane-3-one-18-al (IV) using one equivalent of chromic acid. Compound IV could also be obtained, although in lower yield, by the direct oxidation of dihydroisorubijervine with 1.6 equivalents of chromic acid. No ketoaldehyde could be isolated when two equivalents of chromic acid were used. Reduction of solanidane-3-one-18-al (IV) using the Huang-Minlon modification of the Wolff–Kishner method gave an excellent yield of an oxygen-free base identical in all respects with solanidane (V).

A sample of solanidane for comparison was obtained by the modified Wolff–Kishner reduction of solanidane-3,12-dione (prepared from rubijervine by the method of Sato and Jacobs[2]). It is interesting to note that the Huang-Minlon modification of the reduction gave only solanidane, whereas Sato and Jacobs[2] found that reduction of the disemicarbazone by the normal Wolff–Kishner procedure yielded the alcohol, solanidane-3β-ol, as the major product.

In addition, dihydroisorubijervine was converted to solanidane-3β-ol by the following method. Oxidation of dihydroisorubijervine with one equivalent of chromic acid in 95% acetic acid gave solanidane-3β-ol-18-al (VI) in 70% yield. Reduction of VI by the Wolff–Kishner method gave an 89% yield of solanidane-3β-ol (VII). Its identity was confirmed by comparison of its infrared spectrum with that of an authentic sample and by conversion to the monoacetyl derivative and the corresponding 3-ketone.[7]

Solanidane-18-al was prepared in surprisingly good yield (84%) by oxidation of solanidane-18-ol with chromic anhydride. When this aldehyde or solanidane-3β-ol-18-al was treated with ethyl orthoformate in ethanol–sulfuric acid solution, no formation of an enol ether could be detected and the majority of starting material was recovered. Carbon 21 or carbon 27, therefore, would not appear to be the site of the aldehyde group (and thus the primary hydroxyl) since both positions possess an α-hydrogen and thus should form an enol ether.

Another route originally considered for the

(1) Presented before the Division of Medicinal Chemistry at the 122nd Meeting of the A.C.S., Atlantic City, N. J., September, 1952.

(2) Y. Sato and W. A. Jacobs, *J. Biol. Chem.*, **179**, 623 (1949).

(3) W. A. Jacobs and L. C. Craig, *ibid.*, **148**, 41 (1943).

(4) W. A. Jacobs and L. C. Craig, *ibid.*, **149**, 451 (1943).

(5) W. A. Jacobs and L. C. Craig, *ibid.*, **159**, 617 (1945).

(6) Y. Sato and W. A. Jacobs, *ibid.*, **191**, 63 (1951).

(7) After this manuscript had been prepared for publication, a Communication to the Editor by Rigby and Burn (*Chemistry and Industry*, **27**, 668 (1952)) appeared in which these authors report the conversion of isorubijervine to solanidane-3β-ol by this same method. This portion of our work thus confirms their results.