Summary

1. The synthesis of various phenolic derivatives of phenylpropanolamine has been described.

2. A preliminary report on their physiological activity has been included. It has been found that: (a) the *p*-hydroxyl group increases pressor activity and decreases toxicity to rabbits. (b) Contrary to expectations, the *m*-hydroxyl increases activity at least twice as much as does the *p*-isomer. It also increases toxicity. (c) The phenolic group introduced into the *o*-position decreases the activity and probably increases the toxicity. (d) The *m,p*-dihydroxy derivative is most active and produces an action resembling very strikingly that of epinephrine. Since it has three carbons in the side chain it is also active after oral administration. The two hydroxyls have increased the activity to a greater extent than the toxicity. (e) It is not safe to predict the toxicity of a compound obtained by the introduction, simultaneously, of more than one group (Nos. 7 and 8, Table I).

PHILADELPHIA, PENNSYLVANIA

[CONTRIBUTION FROM THE DEPARTMENT OF RESEARCH IN PURE CHEMISTRY, MELLON INSTITUTE OF INDUSTRIAL RESEARCH]

THE COMPOSITION OF CHERRY GUM

BY C. L. BUTLER AND LEONARD H. CRETCHER

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In a recent paper on the composition of gum arabic[1] the authors pointed out the unsatisfactory state of our knowledge of substances such as pectins, plant gums, mucilages, hemicelluloses and algins, which may be described generically as acid polysaccharides. Numerous papers dealing with the identification of the sugars in these substances have been published over a period of many years. The problem of the acidic nucleus in gums was long ago attacked by O'Sullivan.[2] After hydrolysis of gums arabic, gedda and tragacanth, he isolated the barium salt of a stable organic acid to which, after analysis he assigned the formula $C_{23}H_{38}O_{22}$. Later, Robinson[3] obtained an acid of similar composition from the gum of *Cochlospermum Gossypium*. Until quite recently, it has been generally accepted, on the basis of O'Sullivan's work, that the acidic nucleus of gums is a complex organic molecule of unknown constitution containing twenty-three carbon atoms in combination with hydrogen and oxygen in the ratio indicated above.

[1] Butler and Cretcher, THIS JOURNAL, **51**, 1519 (1929).

[2] O'Sullivan, *J. Chem. Soc.*, **45**, 41 (1884); *ibid.*, **59**, 1029 (1891); *ibid.*, **79**, 1164 (1901).

[3] Robinson, *ibid.*, **89**, 1496 (1906).

During the last decade there has been a decided reawakening of interest in the chemistry of acid polysaccharides and many new and interesting facts have been discovered. The acidity of the carbohydrates studied has been shown to be due to hexuronic acids. Three of these aldehyde sugar acids have been discovered to date, namely, *d*-glucuronic, *d*-galacturonic and more recently *d*-mannuronic,[4] corresponding in configuration to the three commonly occurring hexose sugars. Apparently the uronic acids are not found free in plants, but occur in highly complex molecules, sometimes as polymers of the uronic acid itself as in algin[4] and sometimes in combination with pentose, methylpentose and hexose sugars. The latter class of substances includes pectin, hemicelluloses from wood, etc., plant mucilages and gums, and the soluble specific substances from bacteria.

Results of recent researches by various investigators have not confirmed the opinion of O'Sullivan[2] that a C_{23} acid is the stable nucleus of gums. The work on gum arabic,[1,5] mesquite gum,[6] flaxseed mucilage,[7] cholla gum[8] and gum tragacanth[9] has shown that these substances consist of acidic nuclei to which are attached various sugar units. The stable acidic nuclei of gum arabic, mesquite gum and flaxseed mucilage have been found to be aldobionic acids consisting of glucuronic or galacturonic acid linked to a hexose or a methylpentose residue. The arabinose has been shown to be less firmly attached in the gum acid molecule than the galactose, as it is split off under milder conditions than is the galactose. The results obtained by Norman[9] with gum tragacanth are partially in agreement with those of O'Sullivan, a tribasic acid consisting of three units of an unidentified uronic acid and one of arabinose having been isolated from the mixture obtained on hydrolysis of the soluble fraction of the gum.

Many of the most interesting problems associated with research in this field are suggested by the fact that the soluble carbohydrates elaborated by the pneumococci have the property of reacting type specifically with pneumonia antisera. These serologically specific carbohydrates on hydrolysis also yield aldobionic acids—from each type of bacteria examined—a chemically different one. A carbohydrate capable of reacting with pneumonia antisera has also been prepared by partial hydrolysis of gum arabic.[10]

It is highly probable that the specific nature of each acidic carbohydrate has its origin in the chemical identity of its component acid nucleus. The fact that as far as the studies have gone no botanically different gums have yielded identical acidic nuclei, is substantial evidence in favor of the idea.

[4] Nelson and Cretcher, THIS JOURNAL, **51**, 1914 (1929); **52**, 2130 (1930).

[5] See also Heidelberger and Kendall, *J. Biol. Chem.*, **84**, 639 (1929).

[6] Anderson and Otis, THIS JOURNAL, **52**, 4461 (1930).

[7] Anderson and Crowder, *ibid.*, **52**, 3711 (1930).

[8] Sands and Klaas, *ibid.*, **51**, 3441 (1929).

[9] Norman, *Biochem. J.*, **25**, 200 (1931).

[10] Heidelberger, Avery and Goebel, *J. Exptl. Med.*, **49**, 847 (1929).

This paper consists of a report on experiments with cherry gum. The sample was collected at Montezuma, Indiana, from wild cherry trees and doubtless approaches more nearly a botanically authentic sample than any which could be purchased on the open market.[11] The air-dried material contained 10.75% moisture and about 1.5% ash. The proportions of basic constituents and silica in the ash are shown in Table I.

TABLE I

ANALYSIS OF CHERRY GUM ASH

	%
MgO	15.8
CaO	27.1
K_2O	33.1
R_2O_3	12.0
SiO_2	12.0
Total	100.0

The gum was found to be largely insoluble, the proportion soluble in cold water being only 14.5%.[12] A test for methoxyl by the Zeisel method gave a negative result. Other samples collected near Pittsburgh, Pa., also gave negative results when submitted to this test. A commercial sample, however, was found to have an appreciable methoxyl content.[13] Plum and peach gums also gave positive methoxyl tests.

There can be very slight assurance of the botanical or chemical uniformity of natural products such as the plant gums, if purchased on the open market. The experience of this Laboratory with a sample of commercial cherry gum has emphasized the necessity of using samples of botanical authenticity as starting material in work of this kind. Discrepancies in the results of various workers such as have been noted in researches of Heidelberger and Kendall[5] and this Laboratory[1] on gum arabic are to be expected unless there is some assurance that the same starting material is used by the different workers.

The sample was analyzed for uronic acid, galactose, total pentose,

[11] Wiesner, "Die Rohstoffe des Pflanzenreiches," Verlag von Wilhelm Engelmann, Leipzig, 1927, Vol. I, p. 1010, states that commercial cherry gum is usually contaminated with other fruit gums such as plum and apricot gums.

[12] Sixty-five grams of crude gum was put into 800 cc. of distilled water to which a little toluene had been added, and allowed to stand with occasional shaking for two days. The top liquor was decanted, more water was added, the mixture was shaken thoroughly and allowed to stand for several hours, and the top liquor was again decanted. This was repeated several times. The insoluble gum, consisting of slippery jelly-like masses, was finally drained through scrim. The cloudy liquor (about 800 cc.) containing the soluble gum became water clear on centrifuging. The solution was evaporated to dryness on a water-bath.

[13] Results which differed markedly from those herein reported were obtained with commercial cherry gum. These will be reported elsewhere.

arabinose and methylpentose by the usual methods[14] with the results shown in Table II. The figures are presented with the full realization that they may not be strictly accurate. It is believed, however, that they give valuable information on the composition of the gum.

TABLE II

CONSTITUENTS OF CHERRY GUM

	%
Ash	1.3
Galactose	27.7
Total pentose	56.1
Arabinose[a]	31.6
Other pentose calcd. as xylose	24.5
Uronic acid	10.1
Methylpentose	Absent
Mannose	Present

[a] Diphenylhydrazone method.

The presence of galactose was shown by the fact that the gum yielded mucic acid when oxidized with nitric acid of specific gravity 1.15. The melting point of a purified sample was 217–218°. Arabinose was demonstrated by the separation of an insoluble diphenylhydrazone, when a hydrolyzed sample of the gum was treated with diphenylhydrazine in 75% alcoholic solution. It was confirmed by the isolation of a benzylphenylhydrazone melting at 172°. Arabinose benzylphenylhydrazone has been found to melt at 174°.[15] As shown in Table II, a great discrepancy in the values for total pentose by the furfuralphloroglucide method and for arabinose by the diphenylhydrazone method was found. This pointed strongly to the presence of another pentose in the gum. The non-acidic fraction of the mixture obtained on hydrolysis of the gum was therefore tested for xylose. The isolation of the characteristic boat-shaped crystals of cadmium bromide–cadmium xylonate showed the presence of this sugar.

Fifteen grams of sugar liquor containing 10 g. of dry matter was oxidized as described by Hudson and Isbell.[16] The solution of sugar acids was converted to cadmium salts by boiling the liquor, after freeing from barium and benzoic acid, with cadmium carbonate. The solution of cadmium salts was concentrated on a water-bath to 40 cc. and an equal volume of absolute alcohol was added. The alcoholic solution was allowed to stand in the ice box for six days. During this time some white crystalline material separated out. A drop of the liquor on a glass showed many of the boat-shaped crystals, characteristic of cadmium bromide–cadmium xylonate when examined under the microscope; 0.7 g. of colorless crystals, $[\alpha]_D$ +2.7°, was filtered from the liquor. The mate-

[14] Van der Haar, "Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydesäuren," Gebrüder Borntraeger, Berlin, 1920, pp. 63 123, 131.

[15] Ref. 14, p. 167.

[16] Hudson and Isbell, THIS JOURNAL, 51, 2225 (1929).

rial was dissolved in 10 cc. of water and 10 cc. of absolute alcohol was added. Colorless crystals began to separate almost immediately from the solution. These were filtered off when the separation appeared complete. They had practically no rotation. A few drops of the mother liquor on evaporation on a glass showed many of the characteristically shaped crystals even under the low power of the microscope. The liquor was evaporated to 5 cc. and 5 cc. of absolute alcohol was added. After standing for several hours 0.08 g. of colorless crystals was obtained, $[\alpha]_D$ +7.5°.

Other samples of cadmium salt prepared from liquors from which the arabinose and part of the other sugars had been removed by treatment with benzylphenyl- or *p*-bromophenylhydrazine had $[\alpha]_D$ +8.0, +8.5, +9.3°. Hudson and Isbell[16] report the rotation of cadmium bromide–cadmium xylonate as $[\alpha]_D$ +8.8°. C. A. Browne found $[\alpha]_D$ +7.4° ["Inaugural Dissertation," Göttingen, 1901, p. 21].

Methylpentose is probably not a constituent of this gum. While a little alcohol-soluble furfuralphloroglucide was obtained when the methylpentose test was carried out, the alcoholic solution was greenish in color rather than red-brown as is the case when methyl furfuralphloroglucide is present. The isolation and identification of mannose and the identification of glucuronic acid were accomplished while working with the acidic nucleus of the gum. Description of the experiments follows.

When cherry gum was submitted to hydrolysis in $N/2$ sulfuric acid at 90° for five hours, all of the pentose, and practically all of the galactose, were split from the acidic nucleus. The acidic fraction isolated as barium salt was found to have the barium and aldehyde content of a salt of an acid consisting of two uronic acid groups and three hexose sugar residues. That the sugar residues were not pentose was shown by the fact that the salt gave only the amount of phloroglucide calculated from the uronic acid content. All samples of this cherry gum degradation product gave on oxidation with nitric acid (sp. gr. 1.15) small amounts of mucic acid. This substance will be referred to as Salt A.

Two 500-g. batches of crude gum were placed in 5-liter round-bottomed flasks with 2 liters of water. After standing overnight, 500 cc. of water containing 44 cc. of concentrated sulfuric acid was added to each flask. The flasks were then heated in boiling water-baths for five hours. The inside temperature was 90°. The liquors were then cooled and filtered through scrim. To each batch of 2.5 liters of liquor was then added an equal volume of methyl alcohol. After standing for several hours, the liquors were filtered from the separated inorganic sulfates, and were then evaporated on a water-bath at 40° until most of the methyl alcohol was removed. The sulfuric acid was then removed with the calculated amount of barium hydroxide in aqueous suspension. The combined liquors were then boiled for one hour in the presence of barium carbonate.

The incompletely neutralized liquor was concentrated to a thin dark-colored sirup to which methyl alcohol was added until precipitation appeared complete. The crude barium salt was dissolved in water and partially precipitated, the less soluble, dirty black material which first separated being discarded. The main fraction of clean salt was precipitated four times from aqueous solution with methyl alcohol. The weight of purified salt was 30 g. Another batch of 35 g. of barium salt was obtained from the main bulk of sugar liquor after removal of the methyl alcohol, by boiling again for two hours in the presence of barium carbonate. The total yield of salt was thus 65 g.

Anal. Subs., 0.4475: volatile at 100°, 0.0275. Found: moisture, 6.1. Subs., 0.4200: $BaSO_4$, 0.1042. Calcd. for $C_{28}H_{46}O_{24}(CO_2)_2Ba$: Ba, 13.8. Found: Ba, 14.6. Subs., 0.5003 (0.4698 g. dry subs.): $N/10$ I, 10.8 cc. Calcd. for $C_{28}H_{46}O_{24}(CO_2)_2Ba$: CHO, 2.9. Found: CHO, 3.3. $[\alpha]_D$ −1.7°.

It is believed from our analytical data that the acid of this salt is made up, for the most part, of a complex of five units, three of which are mannose, and two uronic acid. These constituents are linked together in such a way that the two carboxyl groups of the uronic acids and one aldehyde group, probably of a sugar, are free. It was not possible to carry on the hydrolysis to the complete removal of the galactose without also removing some of the mannose.

The acid of Salt A was further hydrolyzed by refluxing its solution in 1 *N* sulfuric acid for four hours. The acidic fraction was again isolated as barium salt, hereafter called Salt B.

Thirty grams of the five unit barium salt was dissolved in 200 cc. of 1 *N* sulfuric acid to which was added 3.3 g. of concentrated sulfuric acid to make up for the acid removed by the barium. The mixture was filtered and then boiled for four hours under a reflux condenser. The salt was isolated and purified as described above. The yield was 18 g.

Anal. Subs., 0.2780: $BaSO_4$, 0.0728 g. Calcd. for $C_{22}H_{36}O_{19}(CO_2)_2Ba$: Ba, 16.6. Found: Ba, 16.5. Subs., 0.2010: $N/10$ I, 5.2 cc. Calcd. for $C_{22}H_{36}O_{19}(CO_2)_2Ba$: CHO, 3.5. Found: CHO, 3.7. Subs., 0.3400 (0.323 g. dry subs.): furfuralphloroglucide, 0.0500. Calcd. for $C_{22}H_{36}O_{19}(CO_2)_2Ba$: phloroglucide from uronic acid, 0.050, $[\alpha]_D$ −23°.

Analytical figures for barium and aldehyde checked with the calculated values for a salt of an acid consisting of two uronic acid and two sugar residues so linked that the two carboxyl groups and one aldehyde group are free. This salt gave no mucic acid on oxidation with nitric acid and was therefore free of galactose. It gave the amount of furfuralphloroglucide calculated from the uronic acid content.

The sugar liquor resulting from this hydrolysis experiment was evaporated to a hard gum, the weight of which was 4.5 g. A sample of 0.775 g. gave 0.594 g. of hydrazone when treated in aqueous solution with phenylhydrazine at room temperature in the presence of acetic acid. The substance melted at 192–193° (uncorr.). Crystallization from 70% alcohol, followed by washing with 95% alcohol and ether, did not change the melting point. Mannose phenylhydrazone has been reported to melt at various temperatures between 190 and 200°.

When 0.36 g. of the sugar was treated in 10 cc. of aqueous solution with 0.7 g. of phenylhydrazine and 0.5 g. of glacial acetic acid on a boiling water-bath for one-half hour, an osazone was formed. After washing with 30% alcohol, and acetone, and crystallizing from 70% alcohol, 0.16 g. of bright yellow crystals was obtained. The substance melted at 207–208°. The melting point of glucosazone is 210°.[17]

[17] Ref. 14, p. 214.

When 0.7 g. of the hydrazone was decomposed with benzaldehyde according to the method described by Van der Haar,[18] a sugar having the correct rotation for mannose, $[\alpha]_D$ +13.7°, was obtained. The rotation of mannose is +14.3°.

A similar hydrolysis of the acid of Salt B yielded Salt C and a sugar liquor. The salt on analysis proved to be one of an acid containing uronic acid and sugar residues in the proportion of two acid groups to one sugar group so linked that the two carboxyl groups and one aldehyde group were free. The sugar liquor resulting from the hydrolysis of Acid B gave mannose hydrazone when tested with phenylhydrazine. When the three unit acid of Salt C was submitted to further hydrolysis under similar or more vigorous conditions, no definite reaction product could be isolated.

Fifteen grams of the four unit salt was dissolved in 150 cc. of *N* sulfuric acid, the barium sulfate was filtered off and the solution was refluxed gently for four hours. The products of the reaction were worked up as described above. The yield of salt was 7.4 g.

Anal. Subs., 0.3263: volatile at 100°, 0.012. Found: moisture, 3.7%. Subs., 0.3043: $BaSO_4$, 0.1050. Calcd. for $C_{16}H_{26}O_{14}(CO_2)_2Ba$: Ba, 20.6. Found: Ba, 20.3. Subs., 0.2077 (0.2000 g. dry subs.): *N*/10 I, 5.8 cc. Calcd. for $C_{16}H_{26}O_{14}(CO_2)_2Ba$: CHO, 4.3. Found: CHO, 4.2, $[\alpha]_D$ −22.3.

The acid of Salt C, on oxidation with nitric acid (sp. gr. 1.15) gave a fair yield of acid potassium saccharate. This identifies the uronic acid present as glucuronic.

When 200 g. of cherry gum was submitted to hydrolysis under conditions described by Weinmann[19] for the preparation of glucuronic acid from gum arabic, the reaction product (5 g.) isolated as barium salt was found to consist of the three unit Salt C instead of the barium salt of a uronic acid. After removal of the barium from this salt, it was oxidized with nitric acid, sp. gr. 1.15, under conditions described by Van der Haar[20] for the preparation of saccharic acid. The absence of galacturonic acid was shown by the failure to obtain any mucic acid in this experiment. The presence of glucuronic acid was shown by the fact that 3.0 g. of the gummy acid yielded 0.25 g. of potassium salt in the form of crystals resembling acid potassium saccharate.

Anal. Subs., 0.0819: K_2SO_4, 0.0285. Calcd. for $KHC_6H_8O_8$: K, 15.7. Found: K, 15.6.

Another sample of the gum was partially hydrolyzed in cold 18% hydrochloric acid.[10] A clear, thin solution was obtained only after the mixture stood at room temperature for one week. Two hundred grams of cherry gum yielded 28 g. of a complex acid practically free of pentose.

Anal. Subs., 0.3785: volatile at 100°, 0.0135. Found: moisture, 3.6. Subs., 0.4728 (0.4558 g. dry subs.): ash, 0.0005. Found: ash, 0.1. Subs., 1.003 (0.967 g. dry subs.): *N*/10 NaOH, 10.8 cc. Calcd. for $C_{66}H_{108}O_{58}$: equivalent weight, 914; uronic acid, 21.2. Found: equivalent weight, 895.4; uronic acid, 21.6. Subs., 0.5186 (0.5000 g. dry subs.): insoluble phloroglucide, 0.0530. From this value was deducted

[18] Ref. 14, p. 228.

[19] Weinmann, *Ber.*, **62**, 1637 (1929).

[20] Ref. 14, p. 100.

0.0358 g. to allow for the uronic acid present. The remainder, 0.0172 g. corresponds to 0.02 g. of pentose. Calcd. for $C_{66}H_{108}O_{58}$: pentose, none. Found: pentose, 4.0. Subs., 1.013 (0.977 g. dry subs.): mucic acid, 0.3800, corresponding to 0.525 g. of galactose; subs., 1.019 (0.982 g. dry subs.): mucic acid, 0.3955 g., corresponding to 0.544 g. galactose. Calcd. for $C_{66}H_{108}O_{58}$: galactose, 57.2. Found: galactose, 53.7, 55.4.

A sample of this substance was submitted to Dr. Robert Koch, Institute of Pathology, The Western Pennsylvania Hospital, Pittsburgh, Pa., for serological examination. The material did not precipitate anti-pneumococcus sera, types I, II or III. It differs in this respect from the partially hydrolyzed arabic acid prepared by Heidelberger, Avery and Goebel[10] by a similar method from gum arabic.

These experiments show that cherry gum is similar to others in the relative firmness of attachment of the sugar constituents. The pentose residues are the first to be split off, leaving a complex acid containing glucuronic acid, mannose and galactose. Next in order of ease of removal comes galactose, removal of which leaves a complex containing only glucuronic acid and mannose. The acidic nucleus of cherry gum, however, appears not to be similar to the nuclei of many other gums studied, since it contains two units of uronic acid combined with one sugar group instead of one acid with one sugar residue as is found in the aldobionic acids.

By making use of the information gained from the experiments, an approximate picture of the cherry gum molecule and its complex degradation products may be shown, even though analytical data for mannose are lacking. Since no substance was isolated in which uronic acid was united to any other sugar but mannose, it would seem safe to assume that all the mannose and all the uronic acid are accounted for in the five unit acid (Salt A). The whole gum contains 10.1% of uronic acid and we may, therefore, allow for the mannose content, about 15%. This makes the total of all the constituents 110.2%, a normal value for a carbohydrate of this nature. The molecular proportions of the various constituents would thus be arabinose 8, xylose 6, galactose 6, mannose 3, glucuronic acid 2.

By making a similar assumption for the complex acid obtained by hydrolysis of cherry gum in 18% hydrochloric acid, we arrive at the value of about 30% for the mannose content of this substance. The molecular proportions in this complex are, galactose 6, mannose 3, uronic acid 2. The theoretical molecular weight assuming ten molecules of water to be split from the eleven sugar residues is 1828, which agrees well with the value 1791, found by titration with alkali.

Summary

Arabinose, xylose, galactose, mannose and glucuronic acid have been identified as the products of the degradation of cherry gum by hydrolysis in acid solution.

PITTSBURGH, PENNSYLVANIA