

viously, even in this extreme case the urethane can readily be separated from the di-substituted urea, using cold ethylene dichloride as the solvent.

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THE PREPARATION OF THE MANNONIC LACTONES FROM THE SEEDS OF THE DATE PALM (PHŒNIX DACTYLIFERA) AND A STUDY OF THEIR ACTION AND THAT OF NEW DERIVATIVES UPON GASTRIC MUCIN SMEARS.*,**

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The sugar lactones have been known for many years (1, 2), but it is only within comparatively recent times that they have assumed scientific and industrial importance.

From the viewpoint of theoretical organic chemistry, these compounds have been of great importance, since it was through them that the structure of the monosaccharides was proved. Also, the mechanism of the oxidation of the monosaccharides was not clearly understood until the properties of the lactones themselves had been determined.

The pharmaceutical uses of the sugar lactones include the substitution of gluconic acid for part of the citric acid in the preparation of Solution of Magnesium Citrate (3). It is said to produce a stable solution without the disagreeable sour taste of the pharmacopœial solution.

The calcium salts of the aldonic acids are used in calcium therapy and have been shown to be readily absorbed when administered orally (4). Calcium gluconate, included in the U. S. P. XI, has the advantage of being practically tasteless; non-irritant by hypodermic or intramuscular injection; and its intravenous toxicity is about a fourth that of calcium chloride (5).

In an investigation having for its purpose the determination of the relative merits of various dentifrices, Nichols, Hatton and Doherty (6) studied the effect of

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galactonic lactone on gastric mucin smears and found it to be superior to alkalis, soaps and most organic acids for the removal of mucin from glass slides. The removal of mucin from the teeth is important in preventive dentistry because fermentable food particles thoroughly mixed with salivary mucin and impacted between teeth or into pits and fissures against susceptible surfaces are, without doubt, an important factor in the formation of dental caries. As a result of this work, galactonic lactone has been incorporated into tooth pastes and mouth washes as a mucin solvent.

The source of galactonic lactone, which was the western larch, was being rapidly depleted, and a new source was sought. There appears at present to be no commercial utilization of the seeds of the date palm, of which large quantities are available from the preparation of seeded, sugared dates. These seeds have been reported by Tschirch (7), who states that they contain much hemicellulose which is composed of galactan and mannan and gives galactose and mannose on hydrolysis. Schulze, Steiger and Maxwell (8) report that the seeds, upon hydrolysis, liberate mannose and apparently also galactose.

Quantitative determinations of mannan and galactan were made in the laboratory. The seeds were found to contain 48.85 per cent of mannan and only 0.68 per cent of galactan. Obviously date seeds could not be used as a source of galactonic lactone. It was thought probable, however, that mannonic lactones and derivatives could be prepared and substituted for galactonic lactone as a mucin solvent.

The ethanolamines are organic bases of mild alkalinity and have been extensively used as emulsifying agents, to which use they are admirably suited. For these reasons it was thought that amides prepared from ethanolamines and mannonic lactone would be excellent substances for the removal of mucin since they would add the emulsifying properties of the ethanolamine to the lactone.

A few statements concerning the formation and behavior of lactones are given here to clarify the experimental work. The oxidation of sugars takes place through the oxide-ring structure with the direct formation of a lactone (9, 10, 11). Normal sugars yield first the δ -lactone (12, 13, 14). This δ -lactone is unstable in water and an equilibrium is rapidly established between the δ -lactone and the free acid. The free acid is then gradually converted to the γ -lactone, a reaction which has been shown to be irreversible at 20° (15).

EXPERIMENTAL.

Ash determinations on the ground seeds were made in the usual manner with the following average results:

Total ash—1.01 per cent

Water-insoluble ash—0.74 per cent

Water-soluble ash (by difference)—0.26 per cent

Alkalinity of water-soluble ash—0.26 cc. of 0.1N HCl were required to neutralize the water-soluble ash from one Gm. of seed.

Alkalinity of Water-Insoluble Ash.—1.04 cc. of 0.1N HCl were required to neutralize the water-insoluble ash from one Gm. of seed.

When extracted with petroleum benzine, date seeds yielded 8.4 per cent of a light yellow oil.

Quantitative Determination of Mannan (16).—Date seeds were hydrolyzed by boiling with dilute acid and the mannose formed precipitated as the phenyl hydrazone. The yield was equivalent to 54.23 per cent of mannose, or 48.85 per cent of mannan.

Quantitative Determination of Galactan (17).—The galactan of date seeds was oxidized by means of nitric acid to mucic acid, which was crystallized and weighed. The quantity obtained was equivalent to 0.75 per cent of galactose, or 0.68 per cent of galactan.

Preparation of Mannose (18).—Mannose was prepared with a yield of 21.1 per cent of the date seeds used.

Preparation of Calcium Mannonate.—The method employed was similar to that of Isbell and Frush (19); however, instead of vegetable ivory, date seeds were used as the starting material. The only innovation was in the use of milk of lime and calcium carbonate to neutralize the acid used in hydrolysis. By this method, neutralization may be accomplished in less time than when calcium carbonate alone is used. Ninety-three grams of calcium mannonate were obtained from 300 Gm. of ground date seeds.

Some of the usual methods for inducing crystallization failed when applied to the solution of calcium mannonate. Concentration resulted in the formation of a gel. The addition of a small amount methanol, ethanol, dioxan or acetone produced colloidal precipitates. The addition of large amounts of methanol produced a hard mass, which, after innumerable washings and the expenditure of enormous quantities of methanol, was transformed to a white solid. This solid, however, could not be entirely freed from bromides, nor was it crystalline, and, therefore, could not be used for seed. Seed crystals were obtained from the National Bureau of Standards, and it was found that crystallization took place readily when the solution was seeded and not too concentrated.

Preparation of δ - and γ -Lactones.—A dry mixture of 40 Gm. of calcium mannonate and 10.6 Gm. of crystalline oxalic acid was added, with mechanical stirring, to 40 cc. of water. The resulting solution was filtered, an equal volume of dioxan added and the solution evaporated rapidly at 35° under reduced pressure. As the solution became more concentrated, δ -mannonic lactone crystallized, and three fractions were collected:

1. 5.8 Gm., melting at 163–165°
2. 4.8 Gm., melting at 159–161°
3. 3.4 Gm., melting at 159–161°.

A few drops of hydrochloric acid were added to the mother liquor and the solution evaporated to dryness. The crude lactone was recrystallized from water and gave 8 Gm. of pure γ -lactone, melting at 151°.

The specific rotation of the γ -lactone was found to be $[\alpha]_D^{20} = +51.00^\circ$. This rotation did not change appreciably after several days at room temperature.

A solution of 0.4207 Gm. of the δ -lactone in 9.8933 Gm. of water gave the following rotations:

Time after Solution.	Rotation.	Specific Rotation.
4 min.	4.73°	114.2°
6 hr.	2.63°	63.5°
24 hr.	1.35°	32.6°
48 hr.	1.40°	33.8°
336 hr.	1.71°	41.3°

These results, which agreed approximately with those previously reported, indicated the establishment of an equilibrium between the δ -lactone and the free acid within 24 hours after solution. At this time there appeared to be about 28.5 per cent of δ -lactone and 71.5 per cent of free mannonic acid present in solution. The rotation was constant for several hours and then increased gradually until it approached that of the γ -lactone.

Derivatives of mannonic acid were prepared for the purpose of characterization and also with a view toward testing their action upon mucin smears. These derivatives were of three types: ethers, esters and amides.

The preparation of a phenyl ether was attempted. It was considered possible that sodium phenoxide would open the lactone ring, the phenyl group going to form a phenyl ether and the sodium going to form the sodium salt. Different attempts were made with benzene and with toluene as the solvent. An excess of sodium phenoxide was prepared, δ -gluconolactone was added, and the mixture refluxed. The product was precipitated as the calcium salt. The per cent of

calcium in this salt indicated that it contained at best about twenty per cent of the calcium salt of phenylgluconic acid.

Several esters of mannonic and gluconic acids were prepared by this procedure: The lactone was heated with the appropriate water-free alcohol, to which a drop of hydrochloric acid had been added. When the lactone was completely dissolved, the solution was filtered, concentrated and cooled to allow crystallization to take place.

Ethyl mannonate was prepared, melting at 161°. The specific rotation was zero, but this slowly increased until after two weeks it had become $[\alpha]_D^{20} = +31.9^\circ$. From these results it was concluded that ethyl mannonate is optically inactive and that in aqueous solution it is slowly hydrolyzed. Because of the slowness of the hydrolysis the end-product, in addition to ethyl alcohol, is undoubtedly the γ -lactone.

Methyl mannonate was prepared, melting at 155°. The specific rotation was found to be $[\alpha]_D^{20} = +11.1^\circ$. The rotation increased slowly until after 15 days it corresponded to $[\alpha]_D^{20} = +44.7^\circ$.

Isopropyl mannonate was prepared, melting at 169°.

n-Butyl mannonate was also prepared, melting at 144°.

Esters of gluconic acid have much lower melting points than the corresponding esters of mannonic acid and become gummy at relatively low temperatures. They would be difficult to preserve in the solid state and, therefore, no further work was attempted with these substances.

To prepare the ethanolamide of mannonic acid, one Gm. of δ -mannonic lactone and 0.4 Gm. of ethanolamine were refluxed with 50 cc. of alcohol until solution was effected. The filtered solution was concentrated and cooled; whereupon crystallization took place immediately. A yield of 0.9 Gm. of a white, crystalline compound was obtained melting at 149–150°. The per cent of nitrogen, as determined by the Kjeldahl method, was found to be 5.76 per cent; calculated, 5.86 per cent. The specific rotation was found to be $[\alpha]_D^{20} = -14.4^\circ$. In 48 hours, the rotation corresponded to $[\alpha]_D^{20} = -12.2^\circ$. The amide may be concluded to be relatively stable in water.

The ethanolamide of gluconic acid was prepared similarly. Transparent, hard crystals were obtained, melting at 109°. The optical rotation was found to be $[\alpha]_D^{20} = +34.15^\circ$. The rotation did not change after four days, showing that the amide is stable in aqueous solution at room temperature.

The diethanolamide of mannonic acid was prepared similarly. It was found, however, that from concentrated solutions the amide separated as a syrup, but that from dilute solutions a white amorphous solid gradually precipitated as the solvent evaporated spontaneously. This solid melted at 123°. The specific rotation of this amide was found to be $[\alpha]_D^{20} = +5.85^\circ$.

The diethanolamide of gluconic acid was obtained only as a syrup. No amount of manipulation would give a solid compound.

TESTS ON MUCIN SMEARS.

Preparation of Mucin Smears.—One Gm. of Wilson's alcohol-washed mucin was placed in a beaker and to it added, with stirring, 15 cc. of a freshly prepared solution of sodium bicarbonate. The resulting mucinate, which had a p_H approximately that of saliva, was allowed to stand in the refrigerator until all air bubbles had been removed.

The films were made on ordinary microscope slides. The slides were first cleaned by soaking in cleansing solution (potassium dichromate-sulfuric acid), rinsed thoroughly with tap water then distilled water, placed in alcohol and dried with a towel. One-tenth of a cc. of the mucin mixture was measured with a graduated medicine dropper and spread on the slide over an area of 2" by $\frac{5}{8}$ ". The film was allowed to dry in air and a control slide made.

The mucin film was immersed in a saturated solution of mercuric bichloride for one minute. The slide was removed and washed with distilled water to remove the mercury salt and to determine whether the film adhered. The mercury bichloride fixed the mucin film firmly to the glass slide, and the film of the control slide was not removed when washed. The film was stained in a 0.5 per cent solution of malachite green. The film was washed with distilled water and allowed to dry.

In determining the effect of chemicals on the mucin film, the following method was used. The tooth brush, a Dr. Butler's single row tooth brush, was moistened with the solution of the chemical and stroked downward through the film three times, the pressure being the same as i

used in the cleansing of the teeth. The slide was immediately placed in the solution of mercury bichloride to fix the film, and then washed with distilled water. The film was stained and photographed. The original method of Nichols, Hatton and Doherty was deviated from slightly in that the slides were washed before staining, since it was found that malachite green was precipitated by mercury bichloride.

The effect of alkaline substances was determined. When mucin smears were treated with a suspension containing equal quantities of milk of magnesia and water, the mucin was disturbed but not removed. A strong solution of Castile soap and five per cent sodium hydroxide solution gave similar results. The alkali appeared to make the mucin more viscous, and it adhered more tenaciously to the slide than it did before treatment. Lime water removed some of the mucin, although the effect was not appreciably greater than that of distilled water alone.

Acidic substances, on the other hand, removed mucin quite completely. A 0.5 per cent solution of citric acid removed mucin completely, while a 0.5 per cent solution of tartaric acid was only slightly less efficient.

The sugar lactones were next investigated, three per cent solutions being used. Of the γ -lactones, galactonic was better than either gluconic or mannonic. δ -Mannonic lactone removed nearly all of the mucin, and the equilibrium mixture of δ -lactone and free acid did remove all.

Derivatives of mannonic acid were also tested. Ethyl mannonate was not as efficient as the ethanolamide and diethanolamide.

The results shown here are essentially the same as those obtained by the originators of the testing method. However, the conclusions arrived at by these workers are erroneous in that they apparently failed to take into consideration the reaction between the materials tested and the mercury bichloride. Alkaline and saponaceous substances react with mercury bichloride with the formation of the corresponding mercury salt. This mercury salt, being adhesive and insoluble in water, remains on the slide and is not removed in the regular process of washing. This film, when placed in the malachite green solution, takes up the stain, making the slide appear as though little or no mucin had been removed.

The apparent success of the acid solutions in removing mucin, as shown by this method, is explained by the fact that citric, tartaric, lactic and acetic acids and the lactones tested form soluble compounds with mercury, and these are removed from the slide by washing. Furthermore, the acid solutions prevent the mercury from fixing the mucin as mercury mucinate. Consequently, the mucin is almost entirely removed from the slide during the process of washing.

In order to determine whether the amides which had been prepared could safely be used in cleansing the teeth, toxicity tests were conducted. Rats were used as the test animals. The dose was calculated at four Gm. per Kg. of body weight and was injected intraperitoneally in ten per cent solution. Neither the ethanolamide nor the diethanolamide produced any ill effects, and it may be concluded that they are non-toxic.

SUMMARY.

1. The mannonic lactones have been prepared in good yield directly from date seeds, thereby establishing the possibility of date seeds as a commercial source of mannonic lactones.

2. Esters, ethanolamides and diethanolamides of gluconic and mannonic acids have been prepared and characterized.

3. The efficiency of mannonic lactones and amides as a solvent for mucin has been shown to be equal to that of galactonic lactone and organic acids, according to the method of Nichols, Hatton and Doherty.

4. It has been pointed out that, in the above method, alkaline and saponaceous substances react with mercury bichloride with the formation of insoluble, adhesive compounds; that acidic substances, including the lactones, form soluble mercury compounds; and that acidic substances prevent the fixing of mucin as mercury mucinate.

5. The ethanolamide and diethanolamide of gluconic acid have been shown to be non-toxic, even in massive doses.

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A STUDY OF ANTISEPTIC COMPOUNDS FOR THE TREATMENT OF BURNS.*¹

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INTRODUCTION.

The anesthetic properties of cocaine were noted in 1884, twenty-four years after the discovery of the alkaloid was announced (1). As a local anesthetic it has never been entirely acceptable because it often produces untoward effects. Since the determination of its structure many synthetic substitutes have been prepared and introduced into Medicine and Pharmacy.

The idea of reacting a local anesthetic with an antiseptic to form a new compound having both anesthetic and antiseptic action was first reported by Thayer (2) who prepared such a compound from butesin (normal-butyl-para aminobenzoate) and picric acid. His preparation represented a combination of one mole of picric acid and two moles of butesin and had the antiseptic and anesthetic properties of these respective compounds. Butesin picrate in the form of an ointment has been widely used in the treatment of burns.

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