TABLE II

Run	Compound	°C.	Successive constants × 10⁴					Average *k*
2	$\underbrace{CH_2CH_2CH}C(=NH_2Cl)OC_2H_5$	25	16.3	17.8	17.4	18.7		0.00176
3		0	0.939	1.054	0.968	1.003		.0000991
4	$\underbrace{CH_2CH_2C}(C_6H_5)C(=NH_2Cl)OC_2H_5$	25	29.7	29.1	26.9	24.2	23.8	.00265
5		25	25.7	24.5	25.0	23.8		.00247
6		25	25.7	24.0	21.8	19.0		.00226
7	$\underbrace{CH_2CH(CH_3)C}(C_6H_5)C(=NH_2Cl)OC_2H_5$	25	17.2	14.3	12.6			.00147
8		25	16.8	12.6	13.3	11.6		.00136

shaken, the carbon tetrachloride layer was drawn off, and the extraction was repeated twice with fresh portions of this solvent. Finally the residual aqueous alkaline solution was titrated with 0.1 *N* hydrochloric acid. Table I gives the data for a typical run.

Table II presents a summary of runs similar to that recorded in Table I.

In view of the fact that compound (II) appeared to be relatively pure, the drift in the values of the constants of runs 4, 5 and 6 suggests a secondary concurrent reaction, although this has not been ascertained experimentally. Runs 7 and 8 on the methylated derivative are of qualitative value only.

Summary

1. The hydrochlorides of ethyl imidocyclopropanecarboxylate and ethyl imido-1-phenylcyclopropanecarboxylate are pyrolyzed normally according to the Pinner–Stieglitz reaction to give the corresponding amides and ethyl chloride. No evidence was obtained of a ring rupture to give pyrrolinium salts.

2. A study of the reaction velocity constants of our imido ester salts with water and those previously determined by Stieglitz and his collaborators for other compounds shows that by this criterion the cyclopropyl radicals should be classified with the electronegative aryl radicals.

Unfinished work on the free esters and amidines will be completed and reported at a later date.

TROY, N. Y. RECEIVED OCTOBER 5, 1936

[CONTRIBUTION FROM THE AVERY LABORATORY OF CHEMISTRY, UNIVERSITY OF NEBRASKA]

Acetyl Derivatives of the Monobasic Sugar Acid Lactones

BY FRED W. UPSON, JOHN M. BRACKENBURY AND CARL LINN

The preparation of a number of acetyl derivatives of the monobasic sugar acid lactones is described in this paper and their properties are compared with those of the parent lactones. Since these derivatives are easily prepared, they are useful in identifying the lactones and in synthesizing other compounds.[1] Furthermore, since no other derivatives have been prepared directly from the δ-lactones the preparation of the acetyl derivatives of these lactones is of interest.

It has been shown[2] that the monobasic lactones having an amylene oxide structure, the δ-lactones, are rapidly hydrolyzed in water solution. Loder[3] found that methylation of *d*-mannonic δ-lactone yielded a derivative having the properties of the γ-lactone. A study of the acetyl compounds was undertaken in an effort to prepare derivatives directly from the δ-lactones[4] and this paper is a continuation of the earlier work of Upson and Bartz. A non-crystalline substance identified as the 2,3,4,6-tetraacetyl derivative was obtained by direct acetylation of *d*-gluconic δ-lactone. This compound though non-crystalline, showed a very rapid mutarotation in 80% acetone–water solution, and was otherwise characterized as a derivative of a δ-lactone.

The acetyl derivatives of thirteen sugar acid lactones have been prepared and their specific rotations have been determined in comparison with those of the parent lactones. The following compounds prepared for this study are already described in the literature: 2,3,5-triacetyl-*d*-xylonic[5] and *l*-arabonic,[6] γ-lactones; the 2,3,5,6-tetraacetyl derivatives of the γ-lactones of *d*-gluconic,[4] *d*-mannonic,[4] α-*d*-glucoheptonic,[4] and α-*l*-rhamnohexonic acids;[7] and 2,3,4,6-tetraacetyl-*d*-gluconic δ-lactone.[4] In addition the following

---

[1] Paal and Kinscher, *Ber.*, **44**, 3543 (1911); **39**, 1361, 2823, 2827 (1906).

[2] Levene and Simms, *J. Biol. Chem.*, **65**, 31 (1925).

[3] Loder, unpublished thesis, University of Nebraska, 1927.

[4] Upson and Bartz, THIS JOURNAL, **53**, 4226 (1931).

[5] Hasenfratz, *Compt. rend.*, **196**, 350 (1933).

[6] Simon and Hasenfratz, *ibid.*, **179**, 1165 (1924).

[7] Miksic, *Vestnik Kral. Ces., Spol. Nauk.* Cl. II, 18 pp. (1926).

new compounds have been synthesized: the 2,3,5,6-tetraacetyl derivatives of the γ-lactones of *d*-galactonic, *d*-gulonic, *d*-talonic and *l*-rhamnonic acids and the 2,3,4,6-tetraacetyl derivatives of the δ-lactones of *l*-rhamnonic and *d*-mannonic acids.

Experimental

Acetylation was accomplished by means of acetic anhydride in the presence of a catalyst. Anhydrous zinc chloride, pyridine and sulfuric acids have been used. The acetylated product is separated from the catalyst by means of water, a process which tends to cause hydrolysis of the lactones. Dry hydrogen chloride in acetic anhydride was found to be a suitable acetylating agent and the possibility of hydrolysis is avoided since excess reagent may be removed by distillation under reduced pressure.

Preparation of the Acetyl Derivative.—Dry hydrogen chloride was passed into acetic anhydride for several minutes. After cooling to 0° the pure lactone was added, 5 cc. of acetic anhydride being used for each gram of lactone. The lactone went into solution at room temperature, the time required varying from a few hours to two weeks, depending upon the lactone used. After the substance had been completely in solution for at least one day the solution was transferred to a distilling flask of appropriate size and the acetic anhydride excess removed under 15–20 mm. pressure. During this distillation the temperature was controlled by a water-bath, the temperature of which was raised slowly to a maximum of 80°. Occasionally the derivative would crystallize during this process. After all acetic anhydride had been removed, the residue was dissolved in chloroform, filtered and the product finally thrown out of solution by the addition of petroleum ether. The product in most cases was solid at this point and was filtered off, dried in vacuum over potassium hydroxide and finally recrystallized from absolute ethanol. Butanol was usedf or the derivatives of *l*-rhamnonic and α-*l*-rhamnohexonic lactones since these are very soluble in ethanol. If the product thrown down by the petroleum ether was non-crystalline, it was washed several times with petroleum ether, the latter removed by heating at 40° under 20 mm. pressure and the resulting liquid kept in a vacuum over potassium hydroxide, with frequent stirring. Various solvents were tried when the product was persistently non-crystalline.

The following new acetylated lactones were prepared using the method above described. In each instance the corresponding pure lactone was used and the acetylated products were characterized by analyses for carbon and hydrogen as well as by their optical properties.

The acetylation of *d*-gluconic δ-lactone using zinc chloride as the catalyst gives a tetraacetylgluconic acid monohydrate as shown by Upson and Bartz.[4] This possesses a melting point of 114–117° and the specific rotation is very nearly zero. On heating in vacuum for some hours at 100° water is given off and there remains a colorless glass-like solid which analyzes correctly for a tetraacetylgluconic lactone. Furthermore, it exhibits the very rapid mutarotation characteristic of δ-lactones. Acetylation according to the method described in this paper using hydrochloric acid as the catalyst produced the same non-crystalline solid which checked in every particular with the 2,3,4,6-acetylated lactone described by Upson and Bartz. The rotation of this substance is recorded in this paper. The acetyl derivatives of γ-gluconic, γ-talonic and γ-rhamnonic lactones are also liquid, but the non-crystalline character of these compounds does not appear to be related to asymmetric structure.

Two acetyl derivatives prepared in this study, namely, 2,3,5,6-tetraacetyl-γ-*l*-rhamnohexonic lactone and 2,3,5,6-tetraacetyl-*d*-talonic γ-lactone possess unusually low initial rotations, and there is little change in the rotation with time. These low rotations are characteristic of free acids rather than of lactone derivatives. Nevertheless, analyses show that these two compounds are acetylated lactones. Of the remaining compounds the acetyl derivatives of galactonic and glucoheptonic lactones are the only ones which possess significantly lower rotations than do the parent lactones. The acetylated *l*-rhamnonic, γ and δ-lactones have rotations somewhat higher than those of the parent lactones whereas the remaining lactones and acetylated derivatives correspond fairly closely as regards specific rotation.

The mutarotation of the lactones and their acetyl derivatives is recorded by means of graphs in Figs. 1 and 2. The graphs in Fig. 2 are plotted without reference to the sign of rotation. The sign of rotation is negative in the case of rhamnonic lactone and its acetyl derivative and is positive for the mannonic and gluconic derivatives.

Time is plotted in *days* in Fig. 1 and in *hours* in Fig. 2 because of the more rapid change of rotation with time in the case of the delta lactones. Polariscopic readings were taken at intervals during a period of several days and the solutions of the various lactones were maintained at a temperature of 25° during this period. Water was the solvent used in the case of the lactones whereas the solvent for the acetyl derivatives was a mixture of acetone and water in the proportions of 80 and 20%.

An inspection of Fig. 1 brings out a number of points. In all cases the sign of rotation of the lactone and its acetyl derivative is the same. In a number of instances the specific rotation of the acetyl derivative is notably less than that of the parent lactone. The derivatives of galactonic (1 and 1a), gulonic (2 and 2a), talonic (4 and 4a) and rhamnohexonic (10 and 10a) fall in this group. In the case

TABLE I

		$[\alpha]^{25}_D$				
Tetraacetyl lactone	M. p., °C.		Min.			Days
2,3,5,6-*d*-Gulonic γ-	103–104	− 37°	5		−35.5°	5
2,3,5,6-*d*-Galactonic γ-	67–68	− 21.7°	13		−19.9°	6
2,3,5.6-*d*-Talonic γ-	Non-cryst. sirup	− 8°	10		− 3°	5
2,3,5,6-*l*-Rhamnonic γ-	Non-cryst.	− 60.1°	20		−57.9°	5
2,3,4,6-*l*-Rhamnonic δ-	71	−113.8°	10	−89.3° 31 hrs.	−40.8°	8
2.3,4,6-*d*-Mannonic δ-	99–101	+ 96.3°	6	+60° 22 hrs.	+31.3°	6

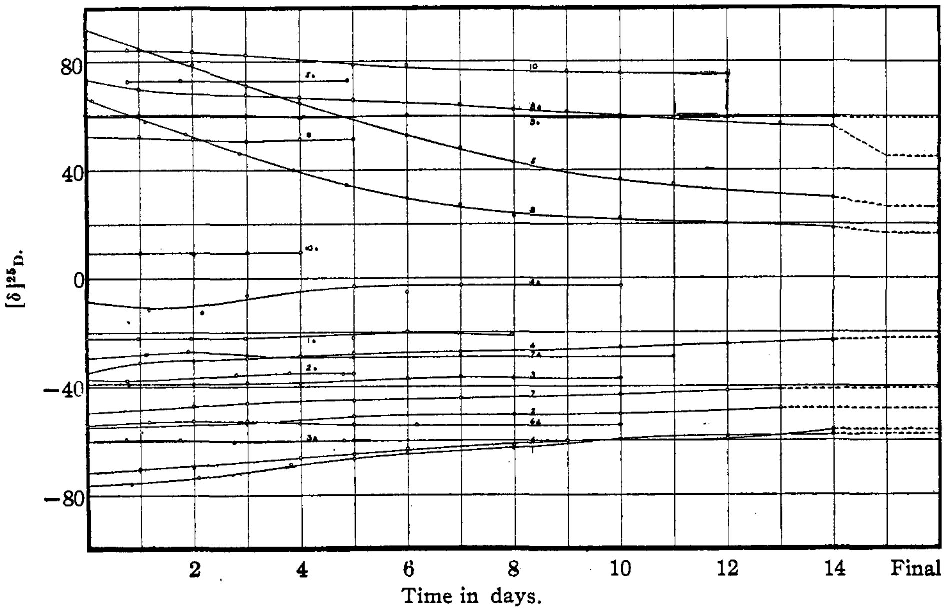

Fig. 1.—Gamma lactones and their acetyl derivatives: 1, 1a, *d*-galactonic; 2, 2a, *d*-gulonic; 3, 3a, *l*-rhamnonic; 4, 4a, *d*-talonic; 5, 5a, *l*-xylonic; 6, 6a, *l*-arabonic; 7, 7a, *α*-*d*-glucoheptonic; 8, 8a, *d*-gluconic; 9, 9a, *d*-mannonic; 10, 10a, *α*-*l*-rhamnohexonic.

of xylonic (5 and 5a), gluconic (8 and 8a) and mannonic (9 and 9a) the acetyl derivatives possess specific rotations somewhat higher than their lactones. In all cases there is a relatively slow change in rotation with time, the lactones and their acetyl derivatives showing a similar behavior. The only exceptions are found in the case of xylonic (5 and 5a) and gluconic (8 and 8a) where the rotation of the lactone changes somewhat more rapidly than that of the acetyl derivative. It is to be noted, however, that in all cases the mutarotation is relatively small in the first forty-eight to seventy-two hours.

In marked contrast is the behavior of the delta lactones and their acetyl derivatives as illustrated in Fig. 2. As has been noted in numerous investigations the specific rota-

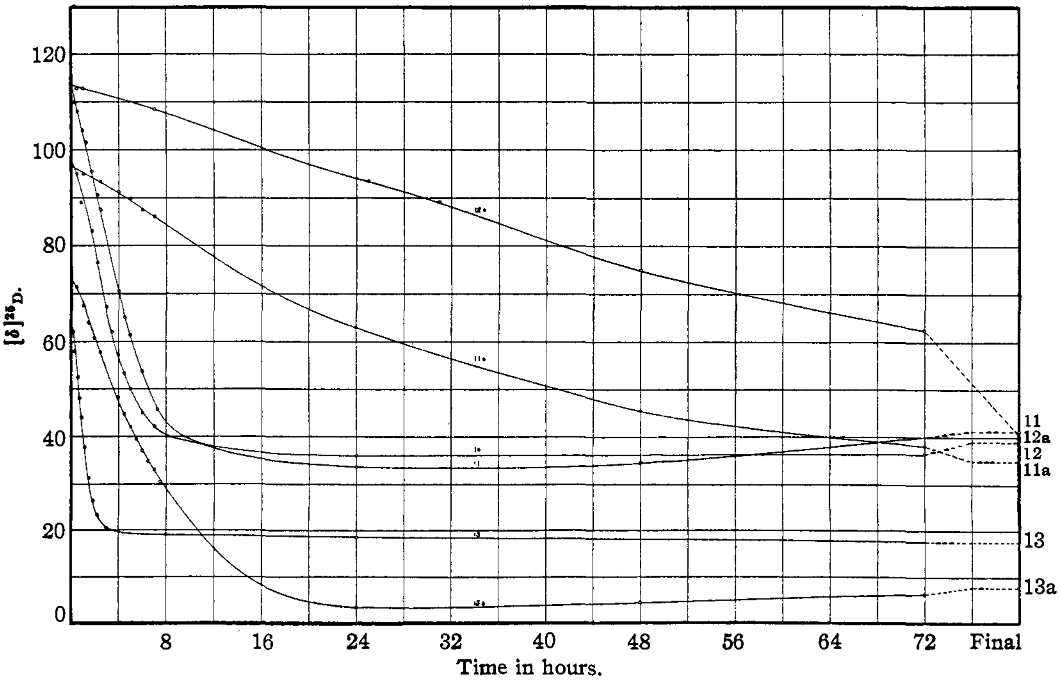

Fig. 2.—Delta lactones and their acetyl derivatives: 11, 11a, *d*-mannonic; 12, 12a, *l*-rhamnonic; 13, 13a *d*-gluconic.

tions of the δ-lactones of gluconic (13), mannonic (11) and rhamnonic acids (12) are relatively high initial specific rotations which show rapid diminution in value during the first few hours after solution. In comparison with the parent lactones, the acetyl derivatives of mannonic (11a) and rhamnonic (12a) do not show as rapid diminution of specific rotation. The behavior of acetyl δ-gluconic lactone, however, parallels very closely that of the parent lactone. In comparison with the corresponding acetyl γ-lactone derivatives these acetylated δ-lactones all show a much more rapid rate of mutarotation and the initial rotations are all very close to those of the parent lactones. Thus the acetylated δ-mannonic lactone (2–11a) changes in rotation from +97 to +63° in twenty-four hours, whereas the corresponding gamma derivative (1–9a) has an initial specific rotation of +60° which shows no change even after ten days. In like manner the rotation of acetylated γ-rhamnonic lactone (2–12a) has an initial value of −114° which changes to −94° in twenty-four hours. while the acetyl derivative of the corresponding γ-lactone (1–3a) exhibits an initial specific rotation of −60° which shows no change even after five days. These acetylated δ-lactones are of special interest since they are the first derivatives to be prepared directly from the delta lactones themselves.

Summary

1. Thirteen acetylated monobasic sugar acid lactones have been prepared, ten of the γ-variety, and three of the δ-configuration.

2. The change in specific rotation with time at 25° has been determined.

3. It has been shown that the acetylated lactones parallel rather closely the parent lactones as regards change of rotations with time.

LINCOLN, NEB.

RECEIVED OCTOBER 8, 1936

[A CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Deuterium Abundance Ratios in Organic Compounds. III. Cholesterol

BY MALCOLM DOLE AND ROBERT B. GIBNEY

The interesting possibility of variations in the isotopic abundance ratios of deuterium in naturally occurring organic compounds as compared to the value of this ratio in normal water has been realized and investigated by a number of workers,[1] but there has not yet been published definite proof of an abnormal deuterium content in any organic compound. The hydrogen of benzene has been analyzed by one of us using a method presumably free of isotopic error[1] but its deuterium content is practically normal. In this paper we report data for the abundance of deuterium in cholesterol which appear to demonstrate the existence of a compound containing a slightly low abundance of deuterium although the observed effect is scarcely greater than the experimental error.[2] We chose cholesterol for this study because it is definitely an animal product,[3] and because its high molecular weight and complexity of structure led us to believe that if any animal product would be abnormal in its deuterium content, cholesterol would be that compound. Cholesterol is also interesting because of its apparently important relations to the hormones and other physiologically active substances. As compared to benzene cholesterol has a relatively high percentage of hydrogen; the small amount of oxygen present introduces a negligible error.

Experimental

Combustion of the Cholesterol.—The method of determining the deuterium content previously used was followed here. The source of the cholesterol was the Wilson Laboratories, Chicago, highest grade "Cholesterin" which comes 95% from the spinal fluid of cattle and 5% from the brains of pigs. The purity of this product was claimed by the manufacturers to be 99.9%, but Fieser states[3] (p. 113) that commercial cholesterol contains small amounts of closely related substances which cannot be eliminated by repeated crystallization, to the extent perhaps of 1 to 2%. Since these related compounds would probably contain similar isotopic proportions of deuterium, this small impurity can be neglected. The melting point temperature of the cholesterol was 149°. The combustion of the cholesterol was carried out using four different methods in order to make it fairly certain that the water obtained contained a representative sample of the hydrogen. Deuterium containing substances apparently burn more slowly than similar compounds made of protium, so that unless all or practically all the cholesterol suffers combustion, the

---

(1) See M. Dole, THIS JOURNAL, 58, 580 (1936). for a review of all previous work and a critical discussion of existing data.

(2) See M. Dole, *Science*, 83, 31 (1936), for calculations showing that the work of Washburn and Smith apparently indicated a smaller than normal abundance of deuterium in dry wood of the willow tree after proper allowance had been made for the difference in atomic weights of oxygen of the air and of water. However, the isotopic composition of the oxygen in the willow tree water is still uncertain even after this correction has been made.

(3) L. F. Fieser, "The Chemistry of Natural Products Related to Phenanthrene," Reinhold Publishing Corporation, New York. 1936, states, p. 122: "Although the evidence is conclusive that the cholesterol present in higher animals is synthesized in the animal organism, the site and mechanism of cholesterol formation are unknown."