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α-Methylene Butyrolactone from *Erythronium americanum*

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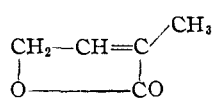
Aqueous extracts of the so-called Adder's Tongue or Dog-Tooth Violet, *Erythronium americanum* (or *dens-canis*) have been observed[1,2] to have a marked growth-inhibitory action against both Gram positive and negative bacteria. The active principle is present in all parts of the plant and may be extracted readily with water, but not with ethanol, ether, ethyl acetate or chloroform. There has been obtained from the plant a hygroscopic, yellow, amorphous solid which exhibits the activity. This substance amounts to approximately 15% of the dry weight of the plant (the fresh plant has about 86–89% moisture) and possesses very low antibacterial activity on a unit weight basis.

Acid hydrolysis of the amorphous principle yielded approximately 10% by weight of a water-soluble skin-irritating liquid of empirical formula $(C_5H_6O_2)_x$. A cryoscopic molecular weight determination showed x to be one. Aqueous solutions were neutral; however treatment with alkali resulted in the liberation of one carboxyl group. Catalytic hydrogenation using an Adams platinum catalyst resulted in the uptake of one mole of hydrogen with formation of a lactone, $C_5H_8O_2$, which was identified as α-methylbutyrolactone (physical properties and hydrazine derivative). The unsaturated lactone did not react readily with bromine water and showed no uptake of halogen from Hanus solution. The latter observations indicated that the double bond was α,β to a carbonyl group.[3,4,5] This conclusion was further supported by the formation on hydrogenation of the saturated lactone rather than of a valeric acid[6] which would have been expected from the β,γ unsaturated isomer. This allows only two possible structures for the natural product



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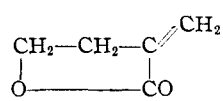
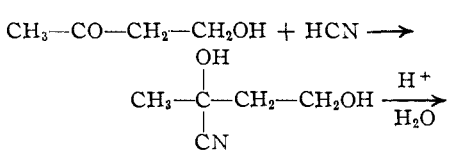
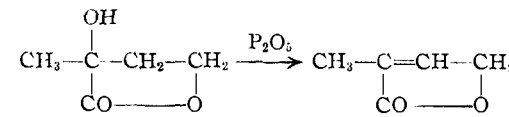


II

Compound I was then synthesized by the sequence of reactions





I

The product obtained was not identical with the natural lactone and showed less than one-tenth the antibacterial activity of the natural product.[7] Whereas lactone I was fairly stable to heat, the natural isomer II polymerized when heated to 70–80° to yield a rubber-like product which became brittle upon standing or with further heating. This tendency to polymerize is characteristic of substances with a methylene group situated as in II.

Spectrographic examination of lactones I and II in water shows no selective absorption in the ultraviolet between λ320 and 220 mμ, showing only end absorption with $\epsilon = 2400$ at λ230 mμ, 900 at λ235 mμ and 500 at λ240 mμ for II; and $\epsilon =$ 900, 270 and 120 at the corresponding λ values for I.

That non-reactivity of the lactone with Hanus solution was a reliable criterion for α,β-unsaturation was demonstrated by investigating the reaction with several types of unsaturated lactones. Although double bonds α,β to a carbonyl group do not react and non-hindered isolated double bonds add one mole of halogen from a Hanus solution, no reference could be found to a reaction of double bonds in enol-aldehyde or enol-ketone lactones or esters with this reagent. It has now been shown that such enol double bonds undergo a reaction with IBr in acetic acid equivalent to utilization of approximately one mole of halogen for every two moles of compound. This was found to be true except when the enol double bond was conjugated to a carbonyl group as in coumalinic acid or dehydroacetic acid with which no halogen was absorbed. The halogen uptake of a series of such compounds is listed in the experimental section. Iodine did not react and bromine added to $\Delta^{\beta,\gamma}$-angelicalactone as has been reported.[8] The differences in the reaction of Hanus solution with conjugated (to carbonyl), isolated and non-conjugated (to carbonyl) enol double bonds may be a useful criterion in the elucidation of certain structures. The mechanism of the reaction was not investigated at this time.

Experimental

Isolation and Properties of α-Methylene Butyrolactone. —Dry *Erythronium americanum* was ground and to 390 g.

(1) Osborn, *Brit. J. Exptl. Path.*, **24**, 227 (1943).
(2) Cavallito and Bailey, *Science*, **100**, 390 (1944).
(3) Thiele and Straus, *Ann.*, **319**, 155 (1901).
(4) Jacobs, Hoffmann and Gustus, *J. Biol. Chem.*, **70**, 1 (1926).
(5) Caldwell and Piontkowski, THIS JOURNAL, **56**, 2086 (1934).
(6) Jacobs and Scott, *J. Biol. Chem.*, **87**, 601 (1930); **93**, 139 (1931).
(7) Antibacterial tests by Dr. John Hays Bailey of these laboratories show I to inhibit completely growth of *Staphylococcus aureus* and of *Bacillus paralyphosus* A in a dilution of 1:200 in broth, whereas the natural lactone inhibits the former organism at 1:2000 and the latter at 1:4000. A comparison of the antibacterial activity of a number of related compounds will appear elsewhere.
(8) Wolff, *Ann.*, **264**, 229 (1891).

was added 3 liters of water (less water required with fresh plant). After standing for one hour the mixture was filtered by suction and the residue was washed with 200 cc. of water. The residue was treated with an additional 2 liters of water and filtered. The combined filtrates totalled 3350 cc.

A 350-cc. aliquot of the extract was concentrated to a sirup under reduced pressure. Pyridine was added in excess to precipitate out inert impurities which were filtered off. The pyridine solution was dried over barium oxide, filtered and dry ethyl acetate was added until no further precipitate was formed. The precipitate was thoroughly washed with dry ethyl acetate and dried at 60° *in vacuo* over paraffin and phosphorus pentoxide, yielding 6.2 g. of hygroscopic, yellow powder which possessed weak antibacterial activity. The yield was 15.2% of the dry plant. An aqueous solution of this product was neutral, but upon addition of standard alkali and back titration with standard acid, one milliequivalent of lactone group was hydrolyzed for 1.095 g. of the amorphous product.

To the 3 liters of remaining extract was added 350 cc. of concentrated hydrochloric acid and the mixture was heated to reflux for thirty minutes. The solution was cooled and extracted three times, each with 600 cc. of chloroform. The chloroform was distilled off and the residue fractionally distilled. Practically all of the material came over at 57–60° at 2 mm. pressure, yield 5.5 g. (this material was collected in May, 1945, and the yield on the basis of dry plant is 1.57%; a lot collected in April, 1946, yielded 2.2% of lactone on the same basis). Distillation of the product at 8 mm. pressure (78–79°) led to formation of considerable polymer.

Anal. Calcd. for $C_5H_6O_2$: C, 61.21; H, 6.16. Found: C, 60.64; H, 6.19.

An aqueous solution of the compound (150 mg. per cc.) had a *p*H of 4 which changed to 7 upon addition of a drop of 0.1 normal alkali. Addition of excess standard alkali and back titration with standard acid showed the presence of a lactone group, molecular weight $(105)_2$; this compares favorably with $C_5H_6O_2$(98). A solution of 0.301 g. of lactone in 13.108 g. of benzene gave a freezing point depression of 1.10°, indicating a molecular weight of 104. Molecular refractivity (Lorentz–Lorenz) on the basis of $n^{20}D$ of 1.470 and d_{20} of 1.1206 is 24.42. The calculated molecular refractivity is 24.32 on the basis[9] of 2.501 for C, 1.051 for H, 1.521 for hydroxyl oxygen, 2.287 for carbonyl oxygen and 1.707 for a carbon to carbon double bond.

The lactone gave a negative Legal test, positive *m*-dinitrobenzene color test,[10] reduced Tollens reagent and neutral permanganate, did not react with bromine water or Hanus solution and gave a negative Schiff test. The lactone apparently has an active unhindered methylene group and is unsaturated α,β to the carbonyl group. Neutral solutions of cysteine or thioglycolate reacted readily to destroy the antibacterial activity of the compound.

The lactone gave a red 2,4-dinitrophenylhydrazine derivative which was crystallized from ethanol, m. p. dec. 190–195°. One molecule of water appears to have been eliminated in the reaction.

Anal. Calcd. for $C_{11}H_{10}O_5N_4$: C, 47.48; H, 3.62; N, 20.14. Found: C, 47.89; H, 3.30; N, 20.27.

Hydrogenation of α-Methylenebutyrolactone.—Catalytic hydrogenation at room temperature of 1.188 g. of lactone in 100 cc. of ethanol with an Adams platinum catalyst resulted in absorption of one molar equivalent of hydrogen in fifteen minutes, no further reduction occurring upon longer exposure. The alcohol was distilled off and the residue was found to distil at 70° at 8 mm. and at 200° at atmospheric pressure; $n^{20}D$ was 1.431. α-Methylbutyrolactone,[11] $n^{24}D$ of 1.4282 has a b. p. of 200–201° at 745 mm.

Anal. of reduction product. Calcd. for $C_5H_8O_2$: C, 59.98; H, 8.06. Found: C, 59.57; H, 7.80.

Treatment of the reduction product with hydrazine hydrate[12] gave a derivative, m. p. 91°.

Anal. Calcd. for $C_5H_{12}O_2N_2$: N, 21.20. Found: N, 21.14.

The derivative of the reduction product gave no depression in a mixed melting point determination with the hydrazide of a synthetic preparation[11] of α-methyl butyrolactone.

α-Methyl-Δα,β-butenolide.—To a cold solution of 133 g. of 95% sodium bisulfite (1.21 moles) in 240 cc. of water was added dropwise with stirring over a period of fifteen minutes, 105 g. (1.19 moles) of 3-ketobutanol.[13] A solution of 90 g. of 95% potassium cyanide (1.3 moles) in 250 cc. of water was added dropwise, in the cold, over a period of thirty minutes. After standing at room temperature for one hour, 600 cc. of concentrated hydrochloric acid was added and the clear solution allowed to stand overnight. The solution was evaporated on a steam-bath and the dark brown residue was filtered from the salt cake. The cake was extracted twice with boiling ethyl acetate. The aqueous filtrate was continuously extracted with ether for twenty-four hours. The ether and ethyl acetate extracts were combined and dried over Drierite. The solvents were removed on a steam-bath and the brown residue was dried *in vacuo* over sodium hydroxide pellets. The oil was distilled under reduced pressure through a Vigreux column. Yield of product distilling at 101–108° at 5 mm. was 69 g. or 50% yield in terms of α-hydroxy-α-methylbutyrolactone. Two more distillations gave a product with b. p. 105–106° at 5 mm., $n^{20}D$ of 1.458 and which was neutral but after treatment with alkali and back titration with acid showed one equivalent of lactone for a molecular weight of 115.

Anal. Calcd. for $C_5H_8O_3$: C, 51.72; H, 6.95. Found: C, 51.47; H, 6.97.

The hydroxylactone upon treatment with *p*-nitrobenzoyl chloride in pyridine yielded a nitrobenzoate, which was crystallized from ethanol, m. p. 162°.

Anal. Calcd. for $C_{12}H_{11}O_6N$: N, 5.28. Found: N, 5.40.

To 10 g. of the hydroxylactone (0.086 mole) in a flask was added, with mixing, 18.5 g. (0.13 mole) of phosphorus pentoxide. Heat was liberated and the mixture formed a sticky mass. Mixing by hand was continued for about twenty minutes, after which heat was no longer liberated. Dry dioxane (50 cc.) was added and, after standing overnight, the mixture was heated on a steam-bath for five minutes. Two layers appeared, the top one was decanted, the residue was washed with dioxane and the combined dioxane extracts were distilled under reduced pressure. The fraction distilling at 74–77° at 5–6 mm. was collected (2.8 g. or 33% yield). This product was dissolved in ether, washed with aqueous sodium bicarbonate solution and the ether dried and evaporated. The product distilled at 75° at 6 mm., $n^{20}D$ was 1.467.

Anal. Calcd. for $C_5H_6O_2$: C, 61.21; H, 6.16. Found: C, 60.85; H, 6.32.

This lactone did not react in a hot ethanolic 2,4-dinitrophenylhydrazine solution, in contrast to the natural isomer.

Preparation of Other Lactones.—The preparations for most of the lactones tested with Hanus solution have been described in a previous paper.[14] The remaining, thiotenol,[15] γ-phenyl-Δβ,γ-butenolide,[16] and coumalinic acid[17] were prepared by literature procedures.

Reaction of Lactones with Hanus Solution.—The iodine number was determined by the usual Hanus procedure and approximately one millimole of lactone was treated for thirty minutes with 25 cc. of Hanus solution containing 2 millimoles of iodine bromide. It was observed that those

(9) Landolt-Börnstein, "Physikalisch-chemische Tabellen," 5th ed., Vol. II, p. 986.

(10) Janovsky, *Ber.*, **24**, 971 (1891).

(11) Adams and Rogers, THIS JOURNAL, **63**, 234 (1941).

(12) Blanc, *Bull. soc. chim.*, [3] **33**, 890 (1905).

(13) White and Haward, *J. Chem. Soc.*, 25 (1943).

(14) Cavallito and Haskell, THIS JOURNAL, **67**, 1991 (1945).

(15) Steinkopf and Thormann, *Ann.*, **540**, 1 (1939).

(16) Kugel, *ibid.*, **299**, 54 (1898).

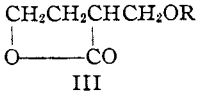
(17) von Pechmann, *ibid.*, **264**, 272 (1891).

compounds which absorbed approximately half-molar quantities of halogen gave a fugitive or temporary end-point in the thiosulfate titration. This was the end-point used in calculating the values given below. When the titrated solution was allowed to stand, the blue starch–iodine color reappeared slowly; however, the rate of reappearance of color was sufficiently slow to allow determination of the end-point.

The quantity of halogen absorbed per mole of lactone according to the procedure described is indicated in parentheses: $\Delta^{\beta,\gamma}$-angelicalactone (0.50), β-methyl-$\Delta^{\beta,\gamma}$-angelicalactone (0.59), α,α-dimethyl-$\Delta^{\beta,\gamma}$-angelicalactone (0.49), α,α,β-trimethyl-$\Delta^{\beta,\gamma}$-butenolide (0.60), α,α,β-trimethyl-$\Delta^{\beta,\gamma}$-angelicalactone (0.27 in thirty minutes reaction time, 0.60 after three days, no further reaction in five days), γ-phenyl-$\Delta^{\beta,\gamma}$-butenolide (0.51), thiotenol (0.46), α-methyl-$\Delta^{\gamma,\delta}$-pentenolactone (0.51) and vinyl acetate (0.53). γ-Methylbutyrolactone, $\Delta^{\alpha,\beta}$-angelicalactone, β-methyl-$\Delta^{\alpha,\beta}$-angelicalactone, coumalinic acid and dehydroacetic acid did not react.

Discussion

The fresh plant contains little if any of the free lactone; however, the amorphous precursor is easily hydrolyzed to yield the lactone. On the basis of lactone content of the precursor as determined by titration, a nearly quantitative formation of α-methylenebutyrolactone results from the hydrolysis if it is assumed that no further lactonization occurs during hydrolysis. The properties are suggestive of a glycosidic structure III



III

in the precursor, which upon hydrolysis may yield the methylene lactone directly or the intermediate β-hydroxylactone. Whether the precursor is active *per se* or depends upon hydrolysis to the methylene lactone in order to exert its antibacterial action is difficult to establish as it may be argued that the microörganism is capable of hydrolyzing the precursor and then is inhibited by the hydrolysis product. The reactivity of the methylene lactone toward thioglycolate adds another example of a non-specific thiol-reactive antibacterial agent.[18,19]

Unsaturated lactones appear to constitute a common type of naturally occurring antibacterial agent as witnessed by the present example, protoanemonin,[20] clavacin,[21] the lactone form of penicillic acid,[22] crepin,[23] and principles of *Arctium minus*[24] and of *Spiraea aruncus*.[25]

A frequent structure among organic antibacterial agents is the $CH_2{=}\underset{|}{C}{-}CO-$ grouping, observed in the present lactone, protoanemonin, penicillic acid and in acrylophenone.[26] Hydrogenation of this group as in penicillic acid[27] destroys the activity and shifting the double bond to give $CH_3{-}\underset{\|}{C}{-}CO-$ as in I greatly reduces the activity. The peculiar virtue of a terminal methylene structure conjugated to a carbonyl group appears to lie in the great reactivity of this structure toward sulfhydryl groups.

Acknowledgment.—We are indebted to Dr. G. W. Ewing for the absorption spectra and to Mr. William F. Warner for routine cylinder-plate antibacterial tests.

Summary

Erythronium americanum yields approximately 15% of dry weight of an amorphous product which upon hydrolysis yields at least 10% of α-methylene butyrolactone. Both the amorphous product and the lactone demonstrate a weak antibacterial action. The mechanism of antibacterial action of this lactone is believed to be similar to that postulated for a number of structurally related antibiotics, and that is by reaction with enzyme —SH groups.

The use of the Hanus iodine number determination as a criterion for elucidating the position of double bonds in unsaturated lactones is discussed.

RENSSELAER, NEW YORK RECEIVED JULY 16, 1946

(18) Cavallito, Bailey, Haskell, McCormick and Warner, *J. Bact.*, **50**, 61 (1945).

(19) Cavallito, *J. Biol. Chem.*, **164**, 29 (1946).

(20) Asahina and Fujita, *Acta Phytochim.* (Japan), **1**, 1 (1922).

(21) Birkinshaw, Bracken, Michael and Raistrick, *Lancet*, **II**, 625 (1943).

(22) Birkinshaw, Oxford and Raistrick, *Biochem. J.*, **30**, 394 (1936).

(23) Heatley, *Brit. J. Expll. Path.*, **25**, 208 (1944).

(24) Cavallito, Bailey and Kirchner, THIS JOURNAL, **67**, 948 (1945).

(25) Abraham, Heatley, Rolt and Osborn, *Nature*, **157**, 511 (1946).

(26) Geiger and Conn, THIS JOURNAL, **67**, 112 (1945).

(27) Oxford, *Chem. Ind.*, **61**, 48 (1942).