improved procedure and nitrosated to give N-methyl-N-nitroso-N′-nitroguanidine.

Neither this nitroso compound nor the parent methylnitroguanidine could be converted to methyldinitroguanidine by a variety of procedures. In each instance methylnitroguanidine was recovered.

When N-methyl-N-nitroso-N′-nitroguanidine is treated with an aqueous solution of an alkylamine, the methylnitrosamino group is eliminated and replaced by an alkylamino group. This provides a convenient method for synthesis of N-alkyl-, N-aryl- and N,N-dimethyl-N′-nitroguanidines.

TORONTO, ONTARIO RECEIVED JULY 1, 1947

[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

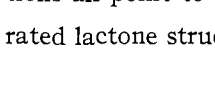
The Antibacterial Principle of *Arctium minus*. II. The Unsaturated Lactone Structure

BY CHESTER J. CAVALLITO AND FRED K. KIRCHNER

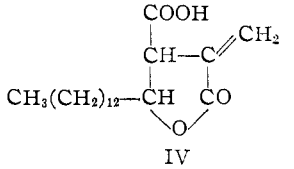
Recently, Abraham, *et al.*,[1] described the isolation and properties of an antibiotic from *Arctium minus* and *Onopordon tauricum* which they believe to be an isomer of the substance (I) isolated by us from the former plant.[2] The empirical formula for both substances is $(C_3H_4O)_x$ in which we had tentatively assigned x as 5; however, later evidence is in agreement with x as 6 in accordance with the Oxford group for their material. Sufficient evidence is now available to indicate the nature of the groups responsible for the antibacterial activity of I.

In order to prevent formation of insoluble amorphous products from I upon standing, it is stored with refrigeration in the form of large crystals. Satisfactory cryoscopic molecular weights could be determined with freshly crystallized I in carefully purified dioxane.

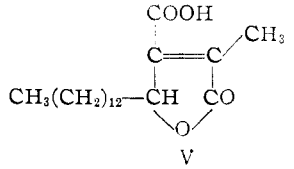
Early work[2] showed that I possessed one double bond reactive toward iodine bromide. Catalytic hydrogenation results in the uptake of two molar equivalents of hydrogen with formation of an amorphous tetrahydro derivative. A second double bond, conjugated to a carbonyl group, has been shown to be present.

The isopropylamine derivative previously described[2] was unsatisfactory for distinguishing between x as 5 or 6 in the molecular formula, hence a corresponding benzylamine derivative, II, was prepared. The nitrogen in these derivatives has basic properties and II formed a relatively water-insoluble crystalline hydrochloride. These basic derivatives show the presence of only one double bond and catalytic hydrogenation results in absorption of one molar equivalent of hydrogen with formation of an amorphous product. Although II is insoluble in sodium bicarbonate solutions, it may be dissolved in cold alcoholic sodium hydroxide. Such solutions, upon dilution with water, do not precipitate II; however, II is formed again upon acidification. These observations indicate that the amine has added to one of the double bonds in I but has not reacted with the lactone group, as evidenced by the basic nature of II and by the remaining lactone structure. The ease of re-lactonization of II upon acidification of an alkaline solution of the substance is evidence in favor of a γ- or δ-lactone, preferably the former. The double bond in I which disappeared with formation of derivative II was the one which did not react with iodine bromide. This indicates it to be α,β to a carbonyl group and is the double bond essential for antibacterial activity, the isopropylamine derivative having no such activity. Whereas I undergoes oxidation and polymerization reactions, II is very stable. These observations all point to the presence in I of an unsaturated lactone structure of type  in which amines would add with the nitrogen attaching to the β- carbon atom of the unsaturated linkage. The prototype of this structure is α-methylene butyrolactone (III) which has been obtained from *Erythronium americanum*.[3] Compound III undergoes polymerization, it readily adds dinitrophenylhydrazine, whereas the isomeric α-methyl-$\Delta^{\alpha,\beta}$-butenolide does not, and the double bond does not add iodine bromide.

Examination of the scientific literature shows that a pair of isomeric unsaturated lactones, protolichesterinic (IV) and lichesterinic (V) acids, also demonstrate considerable differences in ease of addition of amines, such as semicarbazide, IV yielding an addition product involving the double bond whereas V does not react.[4] Samples of IV and V were prepared and it was shown that whereas V does not react, IV yields a crystalline, basic (amphoteric) benzylamine addition product



IV



V

(1) Abraham, Crowfoot, Joseph and Osborn, *Nature*, **158**, 744 (1946).

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

(3) Cavallito and Haskell, *ibid.*, **68**, 2332 (1946).

(4) Asano and Kanematsu, *Ber.*, **65**, 1175 (1932).

as does I. This further favors the type of lactone structure proposed for I.

Although the structure of the remaining portion of the molecule of I has not been determined, several groups are identified. An ester group in addition to the lactone has been demonstrated by strong alkaline hydrolysis of I, as has also been shown by Abraham, *et al.*,[1] for their isomer. Treatment of I with *p*-nitrobenzoyl chloride shows the presence of two esterifiable hydroxyl groups which are not on adjacent carbon atoms (no periodate reaction). The presence of a replaceable hydrogen on the nitrogen of II (indicating that the nitrogen has not taken part in ring formation) was also demonstrated by treatment of II with *p*-nitrobenzoyl chloride, which resulted in three nitrobenzoyl groups entering the molecule. The double bond in I which adds iodine bromide is in an isolated position and not conjugated to either the carbonyl group of the lactone or of the ester portion.[3]

A hydrocarbon has been obtained from zinc-dust distillation of I; however, the structural significance is not yet clear.

The inactivation of I by thiol compounds is readily explained by addition of R–SH to the double bond α,β-to the carbonyl group of the lactone. This indicates the possible mechanism of inhibition of –SH enzymes by I.

Experimental

Molecular Weight of $(C_3H_4O)_x$.—The molecular weight was determined cryoscopically in dioxane with freshly crystallized antibiotic. A freezing point depression of 0.226° was observed with 0.330 g. of antibiotic in 20.44 g. of dioxane, indicating a molecular weight of 335. Where x is six, the molecular weight would be 336.4.

Treatment of the antibiotic with 0.1 *N* sodium hydroxide solution at room temperature resulted in the hydrolysis of one lactone group for an average molecular weight of 317. Heating the compound for one hour with 0.1 *N* alkali resulted in liberation of one equivalent of acid groups for 177 mg., or two acid groups for a molecular weight of 354.

Hydrogenation of $C_{18}H_{24}O_6$.—Hydrogenation was carried out in a modified[5] Parr low pressure hydrogenator. Two molar equivalents of hydrogen was absorbed in thirty minutes by 1.400 g. of antibiotic in 100 cc. of ethanol at 25° with 200 mg. of platinum oxide catalyst and 40 lb. of hydrogen superpressure. Evaporation of the solvent under reduced pressure yielded an amorphous residue which could not be crystallized and was analyzed after drying.

Anal. Found: C, 63.50; H, 8.51. Calcd. for $C_{18}H_{28}O_6$: C, 63.51; H, 8.29.

Isopropylamine Derivative.—This derivative was described in the earlier paper[2] as $C_{18}H_{29}O_5N$ (C, 63.71; H, 8.62; N, 4.13); however, the analytical data fit equally well for $C_{21}H_{33}O_6N$ (C, 63.77; H, 8.41; N, 3.54). Found values for several preparations ranged as follows: C, 63.64–64.35; H, 8.41–8.60; N, 3.68–4.03.

A cryoscopic molecular weight determination with 0.3335 g. of compound in 20.44 g. of dioxane gave a freezing point depression of 0.187°, indicating a molecular weight of 410 ($C_{21}H_{33}O_6N$ mol. wt. 395.5).

Hydrogenation of this derivative under the conditions previously described resulted in absorption of one molar equivalent of hydrogen with formation of an amorphous derivative which could not be characterized.

Benzylamine Derivative.—Treatment of the antibiotic with benzylamine as described for isopropylamine, yielded a derivative which was crystallized as prisms from ethanol, m. p. 157°.

Anal. Found: C, 68.14; H, 7.48; N, 3.41. Calcd. for $C_{25}H_{33}O_6N$: C, 67.69; H, 7.49; N, 3.16.

Addition of hydrochloric acid to a warm ethanolic solution of the derivative resulted in precipitation of a relatively water insoluble crystalline hydrochloride, m. p. 233°. *Anal.* Found: N, 3.18; ionic Cl, 7.48. Calcd. for $C_{25}H_{34}O_6NCl$: N, 2.92; Cl, 7.39.

The benzylamine derivative is insoluble in 2% sodium bicarbonate solution but if dissolved in ethanol and diluted with 10% aqueous sodium hydroxide solution, the precipitate which first appears, redissolves and does not precipitate upon dilution with water. Neutralization of this solution results in precipitation of the benzylamine derivative which shows no depression in a mixed melting point determination with an original sample.

***p*-Nitrobenzoate of Antibiotic.**—To 2 g. of antibiotic in 50 cc. of pyridine was added 5 g. of *p*-nitrobenzoyl chloride and the mixture was shaken for two hours at 25°. Water was added, and the precipitate was filtered off, washed with water, with sodium bicarbonate solution and with dilute hydrochloric acid. The product was crystallized as pale yellow spherules from ethanol containing 10–20% dioxane, m. p. 145°, yield 1.5 g. of pure product. The derivative contains a lactone but no free carboxyl group.

Anal. Found: C, 61.23; H, 5.04; N, 4.51. Calcd. for $C_{32}H_{30}O_{12}N_2$: C, 60.56; H, 4.76; N, 4.42.

This derivative indicates that two hydroxyl groups are present in the original antibiotic.

***p*-Nitrobenzoate of Benzylamine Derivative.**—Two grams of benzylamine derivative was treated with 8 g. of *p*-nitrobenzoyl chloride as described for the previous preparation. The product was obtained from hot ethanol as a pale yellow micro-crystalline substance, yield 3.42 g. When heated slowly, the product shrinks above 100° and fuses from 110–112°.

Anal. Found: total N, Dumas method, 6.11; nitro N, $TiCl_3$ titration, 4.82. Calcd. for $C_{46}H_{42}O_{15}N_4$: total N, 6.29; nitro N, 4.72.

This preparation shows the presence in the benzylamine derivative of two hydroxyl groups and of a replaceable hydrogen on the nitrogen of the benzylamine moiety.

Benzylamine Derivative of *p*-Nitrobenzoate of the Antibiotic.—The bis-(*p*-nitrobenzoate) of the antibiotic (500 mg.) was dissolved in benzylamine and after fifteen minutes water was added and the precipitate filtered off and crystallized as spheroids from alcohol–dioxane solution, yield 300 mg., m. p. 165°.

Anal. Found: total N, Dumas method, 5.50; nitro N, $TiCl_3$ titration, 3.60. Calcd. for $C_{39}H_{39}O_{12}N_3$: total N, 5.66; nitro N, 3.78.

Zinc Dust Distillation of Antibiotic.—A mixture of 3 g. of antibiotic, 15 g. of zinc chloride, 3 g. of sodium chloride and 3 g. of zinc dust was heated slowly to 220°, then rapidly to 290°.[6] A small quantity of colorless oil distilled over which proved to be a hydrocarbon.

Anal. Found: C, 88.25; H, 11.63. Calcd. for $(C_5H_8)_x$: C, 88.17; H, 11.83.

The oil had an odor reminiscent of terpenes, and had an $n^{25}D$ of 1.5432 and d_{25} of 0.9823. No double bonds could be detected by a Hanus iodine number determination. The product may have arisen from interaction of simple C_5H_8 units formed during the degradation.

Reactions of Protolichesterinic and Lichesterinic Acids with Benzylamine.—The *l*-protolichesterinic acid (IV) was obtained from Iceland moss and the *l*-lichesterinic acid (V) was prepared from the former.[4,7] Treatment of 250 mg. of each compound with 2 cc. of benzylamine for fif-

(5) Buck and Jenkins, THIS JOURNAL, **51**, 2163 (1929).

(6) Clar, *Ber.*, **72**, 1645 (1939).

(7) Asahina and Yasue, *Ber.*, **70**, 1053 (1937).

teen minutes at 25° resulted in formation of a derivative of IV, but V was recovered unchanged. The derivative of IV was obtained by diluting the reaction product with water, adjusting the *p*H to 7 and filtering off the precipitate which was then crystallized as white plates from hot dioxane, yield 200 mg., m. p. 177–178°.

Anal. Found: C, 71.95; H, 9.50; N, 3.07. Calcd. for $C_{26}H_{41}O_4N$: C, 72.35; H, 9.57; N, 3.25.

The derivative contained a lactone group and a basic nitrogen (forms insoluble hydrochloride).

Antibacterial and other properties of the lichesterinic acids and related substances will be reported in the near future.

Acknowledgment.—We are indebted to M. E. Auerbach and staff for much of the analytical work presented.

Summary

The antibacterial agent from *Arctium minus*, $C_{18}H_{24}O_6$, has been indicated to have an α-methylene butyrolactone type of structure. The antibacterial properties are attributed to this structural feature. In addition, the compound contains an ester group, an isolated double bond and two hydroxyl groups.

RENSSELAER, NEW YORK RECEIVED JUNE 23, 1947

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK AND CO., INC.]

Streptomyces Antibiotics. XV. N-Methyl-L-glucosamine

BY FREDERICK A. KUEHL, JR., EDWIN H. FLYNN, FREDERICK W. HOLLY, RALPH MOZINGO AND KARL FOLKERS

The degradation of streptomycin resulting in the isolation of a hexosamine and its characterization as N-methyl-L-glucosamine has been reported[1]; the present paper contains a detailed account of these investigations and includes the results on an additional synthesis of N-methyl-L-glucosamine.

The cleavage of streptomycin by the action of methanol in the presence of hydrogen chloride to methyl streptobiosaminide dimethyl acetal hydrochloride and streptidine hydrochloride has been described.[2] Further cleavage of this streptobiosamine derivative is accomplished by the action of concentrated hydrochloric acid at the boiling point for two hours, which results in the decomposition of the nitrogen-free portion, leaving the hexosamine intact.

The hexosamine obtained by hydrolysis was acetylated to give the pentaacetyl derivative as a mixture of the α and β forms. From this mixture, a levorotatory pentaacetyl derivative, m. p. 160.5–161.5°, was obtained by repeated crystallization. This same derivative was obtained in better yield, however, from the crude acetylated product by treatment with zinc chloride and acetic anhydride.[3] Analyses and molecular weight determinations were in agreement with the molecular formula $C_{17}H_{25}NO_{10}$, and showed the presence of five acetyl groups.

Hydrolysis of this acetyl derivative with dilute hydrochloric acid yielded an amorphous hydrochloride which was crystallized from alcohol to give a product melting at 160–163° (dec.). Analyses and results of potentiometric titration were in agreement with the formula $C_7H_{15}NO_5 \cdot HCl$ containing an N-methyl group.[2] This compound reduced both Fehling and Tollens solutions. The hydrochloride was converted into the free base by treatment with silver oxide in methyl alcoholic solution. The free base was acetylated with acetic anhydride in methanol to give the N-acetyl derivative, m. p. 165–166°.

Since all these reactions were in agreement with those expected of a hexosamine, the hydrochloride was treated with phenylhydrazine for phenylosazone formation. A phenylosazone was formed and the methylamino group was eliminated; the reaction required prolonged heating to obtain a good yield. This phenylosazone, which decomposed at 205°,[4] was converted[5] into a phenylosotriazole[1,6] melting at 196–197°. The phenylosotriazole prepared from D-glucose melted at 196–197°; however, the specific rotations of the two phenylosotriazoles were equal in magnitude but opposite in sign. These results indicated that the hexosamine belongs to the L-series and the "natural" phenylosotriazole is phenyl-L-glucosotriazole (I).[1,6]

The position of the methylamino group was demonstrated by oxidation of the hexosamine with mercuric oxide to a nitrogen-containing acid which melted at 230–232° (dec.), the reported melting point of N-methyl-D-glucosamic acid.[7] Again, the rotation was equal in magnitude but opposite in sign, indicating that the acid is N-methyl-L-glucosamic acid (III) and that the hexosamine from streptomycin is N-methyl-L-glucosamine (II).

The conclusions were supported by synthesis of N-methyl-L-glucosamine from L-arabinose by two series of reactions. L-Arabinose (IV) was con-

(1) Kuehl, Flynn, Holly, Mozingo and Folkers, THIS JOURNAL, **68**, 536 (1946).

(2) Brink, Kuehl and Folkers, *Science*, **102**, 506 (1945).

(3) It is assumed that the isomer obtained by the zinc chloride treatment is the α isomer; Gilman, "Organic Chemistry, An Advanced Treatise," 2nd ed., Vol. II, p. 1551, John Wiley and Sons, Inc., New York, 1943.

(4) L-Glucose phenylosazone, m. p. 205°; Fischer, *Ber.*, **23**, 374 (1890).

(5) Haskins, Hann and Hudson, THIS JOURNAL, **67**, 939 (1945).

(6) Wolfrom and Thompson, *ibid.*, **68**, 791 (1946).

(7) Votoček and Lukeš, *Chem. Listy*, **29**, 308 (1935); *Collection Czechoslov. Chem. Commun.*, **7**, 474 (1935).