[CONTRIBUTION FROM THE PRODUCTS DEVELOPMENT LABORATORIES OF PARKE, DAVIS & CO.]

The Structure of Chloramphenicol Palmitate

BY WILLIAM H. EDGERTON, V. HAROLD MADDOX AND JOHN CONTROULIS

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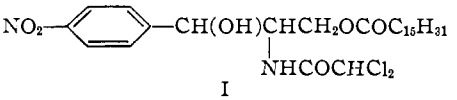
The chromic anhydride oxidation of chloramphenicol palmitate yielded α-dichloroacetamido-*p*-nitroacrylophenone. Chloramphenicol 1-palmitate was prepared by a procedure involving the stereospecific N → O[1] shift of the palmityl moiety.

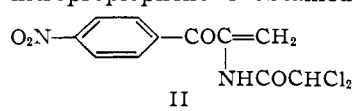
Chloramphenicol[1] has been identified as D-*threo*-2-dichloroacetamido-1-(*p*-nitrophenyl)-1,3-propanediol. Chloramphenicol palmitate[2] has been developed commercially as a tasteless derivative which, upon ingestion, is hydrolyzed to the active antibiotic.



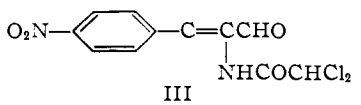
I

Since ester formation generally proceeds more readily in the case of primary hydroxyl groups than in the case of secondary hydroxyl groups, it was presumed that the structure of chloramphenicol palmitate was that shown above (I). This structure has been substantiated by the oxidation of chloramphenicol palmitate, the synthesis of the isomeric 1-ester and acylation studies of a series of oxazolines.[3] This report deals with the oxidation of chloramphenicol palmitate and the preparation of the isomeric chloramphenicol 1-palmitate.

Oxidation of chloramphenicol palmitate with the stoichiometric quantity of chromic anhydride in glacial acetic acid at 50° yielded along with palmitic acid an optically inactive unsaturated ketone, $C_{11}H_8Cl_2N_2O_4$, m.p. 116–117°, identical with the dehydration product of α-dichloroacetamido-β-hydroxy-*p*-nitropropiophenone[4] obtained by Osugi.[5]



II

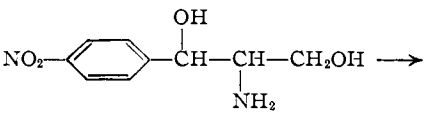


III

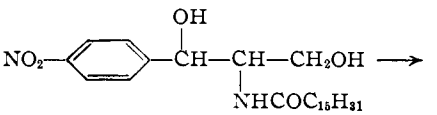
The structure II has been assigned to the unsaturated compound on the basis of: (1) its ultraviolet spectrum (λ_{max} 266 mμ), the isomeric unsaturated aldehyde, structure III, being expected to absorb in the neighborhood of 310 mμ (*p*-nitrocinnamaldehyde, λ_{max} 310 mμ); (2) its absorption of bromine in carbon tetrachloride; (3) the absence of measurable optical rotation indicating loss of asymmetric centers; (4) its hydrolysis in alcoholic hydrochloric acid yielding the expected *p*-nitrophenylpropane-1,2-dione[6]; and finally (5) the identity of the unsaturated compound with Osugi's dehydration product.

Structure II indicates that chloramphenicol palmitate bears the palmityl group on the primary alcoholic carbon atom rather than on the secondary carbon atom. If the original ester had contained the palmityl group on the secondary alcoholic carbon atom, hydrolysis to the alcohol, followed by oxidation and dehydration, would have to be postulated to explain the formation of II. In this case the oxidation of the free primary alcoholic group would have to be very slow to allow the hydrolysis and secondary alcohol oxidation to take place by preference.

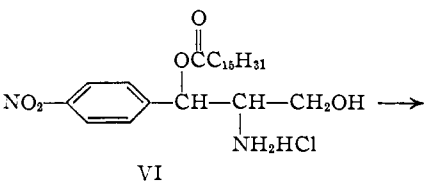
The preparation of the isomeric chloramphenicol 1-palmitate (VII) was then undertaken to confirm the structure and to determine its chemical as well as pharmacological properties.



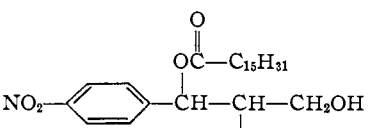
IV



V



VI



VII

Monoacylation of DL-*threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol (IV) by conventional methods resulted in large amounts of O[3], N-dipalmitate, and the Kunz hydrolysis[7] of this diacyl derivative was unsatisfactory because of the solubility characteristics of the ester. A good yield of the N-palmitate (V) could be obtained, however, by using a ratio of two equivalents of IV to one of acid chloride, and this substance was transformed into the secondary monoester VI by a stereospecific shift. Many instances of such reactions have been re-

(1) Chloramphenicol is the generic name of the antibiotic for which the trademark of Parke, Davis and Co. is Chloromycetin.

(2) W. H. Edgerton, U. S. Patent 2,662,906 (December 15, 1953); A. J. Glazko, W. H. Edgerton, W. A. Dill and W. R. Lenz, *Antibiotics and Chemotherapy*, **2**, 234 (1952).

(3) W. H. Edgerton and R. L. Hull, to be published.

(4) L. M. Long and H. D. Troutman, THIS JOURNAL, **73**, 481 (1951).

(5) K. Osugi, *J. Pharm. Soc. Japan*, **72**, 458 (1952); *C. A.*, **47**, 2749 (1953).

(6) V. Petrow, O. Stephenson and B. Sturgeon, *J. Chem. Soc.*, 4066 (1953); L. M. Long, Parke, Davis & Co. Laboratories, unpublished data.

(7) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1982 (1928).

ported.[8] The proximal positions of the 2-acylamino and the 1-hydroxyl moieties enables facile formation of a 5-membered ring as an intermediate according to the plausible mechanism postulated by Alberti and co-workers.[8]

DL-*threo*-2-Amino-1-(*p*-nitrophenyl)-1-O-palmityl-1,3-propanediol was isolated from the hydrochloride VI by shaking in suspension in an alkali–ether system. Although this compound is stable enough to be isolated and characterized,[9] an $O^1 \rightarrow N$ shift occurred when acylation with methyl dichloroacetate was attempted. Acylation of VI was successful with dichloroacetic anhydride in dry benzene.

The preparation of D-*threo*-chloramphenicol 1-palmitate was completed essentially as described above with the exception of the dichloroacylation step. D-*threo*-2-Amino-1-(*p*-nitrophenyl)-1-O-palmityl-1,3-propanediol hydrochloride was acylated in N,N-dimethylformamide with dichloroacetyl chloride. The mechanism of this reaction probably involves the introduction of the dichloroacetyl moiety at the O^3 position. Neutralization then induces an $O^3 \rightarrow N$ migration of the dichloroacetyl group rather than the more sluggish palmityl group.

The infrared spectra of both D- and DL-1-isomers are characterized by distinctive dichloroacetamido absorption bands at 5.93 and 5.96 μ, respectively. Palmitamido absorption usually occurs at about 6.06 μ. The ultraviolet spectra of the D- and DL-1-isomers have peaks which are shifted to 267.5 and 264.5 mμ, respectively.

The authors wish to thank Dr. George W. Moersch for his advice and Mr. James R. Fisher for his assistance. Infrared and ultraviolet spectra were determined and interpreted by Dr. John M. Vandenbelt, Mr. R. Bruce Scott and Miss Carola Henrich. Microanalytical data were applied by Mr. Charles Childs.

Experimental[10]

Oxidation of Chloramphenicol Palmitate.—To a solution of 17 g. (0.0303 mole) of chloramphenicol palmitate in glacial acetic acid (400 ml.) was added 2 g. (0.0200 mole) of chromium trioxide, dissolved in 2 ml. of water and 5 ml. of glacial acetic acid. The mixture was heated at 55° for 16 hours.

The resulting green solution was concentrated *in vacuo* at 55–60° and the residue dissolved in ether and ethyl acetate. After aqueous sodium bicarbonate was added to neutrality, the organic layer was separated and the aqueous layer extracted with ether–ethyl acetate. The combined organic solutions were dried, concentrated, and the residue triturated with petroleum ether. After filtration, 5.4 g. (68.8%) of yellow crystalline material was obtained, m.p. 114–117°. Recrystallization from petroleum ether–ethyl acetate (1:1) gave yellow plates, m.p. 116.5–117.5°, which decolorized bromine in carbon tetrachloride, gave a positive test with 2,4-dinitrophenylhydrazine as well as a negative Schiff aldehyde test, and had an optical rotation of zero in ethanol (*c* 1.33%).

Anal. Calcd. for $C_{11}H_8Cl_2N_2O_4$: C, 43.59; H, 2.66; Cl, 23.40. Found: C, 43.86; H, 2.98; Cl, 23.75.

The 2,4-dinitrophenylhydrazone melted at 236–238°.

Anal. Calcd. for $C_{17}H_{12}Cl_2N_6O_7$: C, 42.25; H, 2.50. Found: C, 42.56; H, 2.92.

A second experiment performed under the same conditions afforded, in addition to the above compound, 5.9 g. of a pale green solid (blue in 2,2,4-trimethylpentane). This material was dissolved in concentrated sulfuric acid to give a deep green solution from which precipitated a waxy, ether soluble solid upon dilution with water. Recrystallization from ether gave 3.1 g. of colorless acid, m.p. 58–60°, identified as palmitic acid by the amide (m.p. 102–104°). In addition, a small quantity (0.16 g.) of *p*-nitrobenzoic acid was isolated from the aqueous solution.

The dehydration of α-dichloroacetamido-β-hydroxy-*p*-nitropropiophenone was carried out by the procedure of Osugi[4] to yield pale yellow crystals, m.p. 116.5–118°, shown to be identical with II which was obtained by the chromic anhydride oxidation of I.

1-(*p*-Nitrophenyl)-propane-1,2-dione.—A solution of 1 g. of α-dichloroacetamido-*p*-nitroacrylophenone in 10 ml. of absolute ethanol and 1.5 ml. of concentrated hydrochloric acid was refluxed for four hours. After cooling the solution to 5°, 1.2 g. of ammonium chloride was separated by filtration. The filtrate was concentrated, dissolved in 10 ml. of ether, filtered and evaporated to dryness. The residue was dissolved in 5 ml. of benzene. After treatment with carbon, the clear solution was diluted with 10 ml. of petroleum ether. On standing, crystals were obtained, m.p. 85–87°. This material was identical with authentic[6] 1-(*p*-nitrophenyl)-propane-1,2-dione as shown by a mixture melting point.

DL-*threo*-2-Dichloroacetamido-1-(*p*-nitrophenyl)-3-O-palmityl-1,3-propanediol.—A solution of 32.3 g. (0.1 mole) of DL-*threo*-2-dichloracetamido-1-(*p*-nitrophenyl)-1,3-propanediol and 9 ml. of dry pyridine in 50 ml. of N,N-dimethylformamide was stirred while 29 g. of palmityl chloride was added in one portion. After stirring for four hours, the reaction mixture was poured into 400 ml. of cold water acidified with 5 ml. of concentrated hydrochloric acid. The crude solid, 55 g. (95%), m.p. 72–79°, was purified by recrystallization from ethanol; m.p. 90–91°, yield 65%.

Anal. Calcd. for $C_{27}H_{42}Cl_2N_2O_6$: C, 57.75; H, 7.54. Found: C, 57.70; H, 7.74.

DL-*threo*-1-(*p*-Nitrophenyl)-2-palmitamido-1,3-propanediol (V).—A solution of 21.2 g. (0.1 mole) of DL-*threo*-2-amino-1-(*p*-nitrophenyl)-1,3-propanediol (VI) in 250 ml. of N,N-dimethylformamide, which had been dried over anhydrous magnesium sulfate, was stirred rapidly while 13.8 g. (0.05 mole) of palmityl chloride was added dropwise. The reaction mixture was stirred at room temperature for four hours and poured into 1 l. of water. The mixture was acidified with dilute hydrochloric acid, then washed well with several fresh portions of water. The tan solid, m.p. 66–69°, was purified by recrystallization from ethanol or ethyl acetate; m.p. 77–78°, yield 65%.

Anal. Calcd. for $C_{25}H_{42}N_2O_5$: C, 66.64; H, 9.40. Found: C, 66.69; H, 9.34.

D-(−)-*threo*-1-(*p*-Nitrophenyl)-2-palmitamido-1,3-propanediol: yield 55%, m.p. 91°, $[\alpha]^{26}D$ −24° (2% in ethyl acetate). *Anal.* Found: C, 66.98; H, 9.21.

DL-*threo*-2-Amino-1-(*p*-nitrophenyl)-1-O-palmityl-1,3-propanediol Hydrochloride (VI).—A solution of 9.0 g. (0.02 mole) of V in 800 ml. of dry ether was saturated with dry hydrogen chloride gas. After several days, 7.3 g. (75%) of white solid, m.p. 133–135°, was isolated. Recrystallization from ethanol or ethyl acetate raised the melting point to 148–149°, yield 20%.

Anal. Calcd. for $C_{25}H_{42}N_2O_5 \cdot HCl$: C, 61.64; H, 8.86. Found: C, 61.34; H, 8.86.

D-(−)-*threo*-2-Amino-1-(*p*-nitrophenyl)-1-O-palmityl-1,3-propanediol Hydrochloride: yield 60%, m.p. 167–168°, $[\alpha]^{26}D$ −24.1° (1.33% in ethanol). *Anal.* Found: C, 62.09; H, 9.21.

DL-*threo*-2-Amino-1-(*p*-nitrophenyl)-1-O-palmityl-1,3-propanediol.—A suspension of 3.2 g. of VI in a system of 100 ml. of saturated sodium bicarbonate solution and 200 ml. of ether was shaken until clear. The organic layer was washed with water, dried over anhydrous magnesium sulfate and evaporated; 1.2 g. (41%) of white solid, m.p. 86–87°. Several recrystallizations from ethyl acetate raised the melt-

(8) C. G. Alberti, B. Camerino and A. Vercellone, *Gazz. chim. ital.*, **82**, 63 (1952); G. Fodor, J. Kiss and I. Sallay, *J. Chem. Soc.*, 1858 (1951); S. Ikuma and R. Myokei, *J. Pharm. Soc. Japan*, **72**, 957 (1952).

(9) This observation is contrary to the behavior of less bulky groups as noted in reference 8.

(10) Melting points are uncorrected.

ing point to 92–93°. The characteristic ester band at 5.81 μ was observed in the infrared spectrum.

Anal. Calcd. for $C_{25}H_{42}N_2O_6$: C, 66.64; H, 9.40. Found: C, 66.77; H, 9.26.

DL-*threo*-2-Dichloroacetamido-1-(*p*-nitrophenyl)-1-O-palmityl-1,3-propanediol (VII).—A suspension of 6.1 g. (0.0125 mole) of VI in 100 ml. of dry benzene with 3.0 g. (0.0125 mole) of dichloroacetic anhydride was heated at reflux for 16 hours. After filtration, the filtrate was evaporated *in vacuo* to a sirup which was taken up in a small volume of isopropyl alcohol and diluted with petroleum ether. After cooling at 5° overnight, 1.3 g. (19%) of white solid, m.p. 70–75°, was recovered. Recrystallization from ethanol raised the melting point to 88–89°. The melting point of a sample admixed with DL-chloramphenicol palmitate was depressed to 80–82°; E^1_1 185 at 264.5 mμ (in ethanol).

Anal. Calcd. for $C_{27}H_{42}Cl_2N_2O_6$: C, 57.75; H, 7.54. Found: C, 57.52; H, 7.23.

D-(−)-*threo*-2-Dichloroacetamido-1-(*p*-nitrophenyl)-1-O-palmityl-1,3-propanediol.—A solution of 9 g. (0.0185 mole) of D–O′ ester in 75 ml. of dry N,N-dimethylformamide was stirred while 3 g. (0.0204 mole) of dichloroacetyl chloride was added dropwise. After standing for 12 hours, the reaction mixture was poured into ether–sodium carbonate solution. The ether layer, after shaking, was separated, dried and evaporated *in vacuo*. The resulting sirup was taken up in hot xylene. Cooling caused the separation of 1.5 g. of the active form of amide V. The filtrate was diluted with petroleum ether and cooled to cause the separation of 1.5 g. (18%) of white solid, m.p. 101–102°. The solid was recrystallized from xylene to a melting point of 105–106°; E^1_1 179 at 267.3 mμ (in ethanol); $[\alpha]^{26}_D$ −39.5° (2% in ethyl acetate).

Anal. Calcd. for $C_{27}H_{42}Cl_2N_2O_6$: C, 57.75; H, 7.54. Found: C, 57.18; H, 7.79.

DETROIT, MICHIGAN

[CONTRIBUTION FROM THE COLLEGE OF PHARMACY, UNIVERSITY OF MICHIGAN]

Antispasmodics. XIX. Esters of 1-Methyl-2-(hydroxymethyl)-pyrrolidine and 1-Methyl-2-(hydroxymethyl)-piperidine

BY F. F. BLICKE AND CHI-JUNG LU[1,2]

RECEIVED MARCH 16, 1954

Salts of the diphenylacetate, benzilate, β-methyltropate and *p*-aminobenzoate of 1-methyl-2-(hydroxymethyl)-pyrrolidine and also the hydrochloride and methobromide of (1-methyl-2-pyrrolidyl)-methyl benzhydryl ether were prepared. The hydrochloride of the *p*-aminobenzoate of 1-methyl-2-(hydroxymethyl)-piperidine was synthesized. The hydrochlorides of the two basic alcohols underwent Mannich reactions. The pharmacological activity of some of the compounds has been reported.

Since a number of esters of basic alcohols, such as β-diethylaminoethanol, are active antispasmodics, it was of interest to prepare esters of 1-methyl-2-(hydroxymethyl)-pyrrolidine (I) and of 1-methyl-2-(hydroxymethyl)-piperidine (II). The basic alcohols I and II contain the same chain skeleton, N–C–C–OH, found in the simpler basic alcohol mentioned above.

In order to obtain the required alcohol I, diethyl glutamate was refluxed in xylene whereby it was converted into 2-carbethoxy-5-pyrrolidone; reduction of the pyrrolidone with lithium aluminum hydride yielded 2-(hydroxymethyl)-pyrrolidine. This pyrrolidine, in the form of its hydrochloride, reacted with formaldehyde and acetophenone to form the hydrochloride of the Mannich base, β-(2-hydroxymethylpyrrolidino)-propiophenone.

When 2-(hydroxymethyl)-pyrrolidine was treated with chloral,[3] the 1-formyl derivative was obtained; this substance was reduced with lithium aluminum hydride to the desired alcohol I.

Alcohol II was obtained in the following manner. α-Picoline was oxidized to α-picolinic acid which, in the form of the hydrochloride, was hydrogenated to α-pipecolinic acid hydrochloride. After esterification and reduction of the ester with lithium aluminum hydride, 2-(hydroxymethyl)-piperidine was obtained. In the form of the hydrochloride, this basic alcohol reacted with formaldehyde and acetophenone to yield the hydrochloride of the Mannich base, β-(2-hydroxymethylpiperidino)-propiophenone. 2-(Hydroxymethyl)-piperidine was converted by chloral into the 1-formyl derivative which was reduced by lithium aluminum hydride to the 1-methyl compound II.

The diphenylacetate of I was prepared by interaction of diphenylacetyl chloride with I. In order to obtain the benzilate, the alcohol I was converted into 1-methyl-2-(chloromethyl)-pyrrolidine (III) which was then allowed to react with benzilic acid according to the Horenstein–Pählicke process.[4] Since basic esters of tropic acid, in the form of salts, are often difficult to obtain in crystalline form,[5] it was decided to employ β-methyltropic acid[6] and to treat this acid with III by the Horenstein–Pählicke procedure. The hydrochloride of the ester formed, (1-methyl-2-pyrrolidyl)-methyl β-methyltropate, was obtained only as an oil but we were able to prepare the crystalline methobromide in poor yield.

Interaction of the alcohol I with diphenylbromomethane produced (1-methyl-2-pyrrolidyl)-methyl benzhydryl ether.

The hydrochlorides of the *p*-aminobenzoates of I and II were obtained by catalytic reduction of the hydrochlorides of the *p*-nitro esters.

The hydrochlorides of the diphenylacetate and the benzilate of I were tested at the Sterling–Winthrop Research Institute on the isolated rabbit in-

(1) This paper represents part of a dissertation submitted by Chi-Jung Lu in partial fulfillment of the requirements for the Ph.D. degree in the University of Michigan, 1952.

(2) American Foundation for Pharmaceutical Education Fellow.

(3) F. F. Blicke and Chi-Jung Lu, THIS JOURNAL, **74**, 3933 (1952).

(4) H. Horenstein and H. Pählicke, *Ber.*, **71**, 1644 (1938).

(5) Unsuccessful attempts have been made in three different laboratories to obtain a crystalline salt of β-diethylaminoethyl tropate. See J. von Braun, O. Braunsdorf and K. Rath, THIS JOURNAL, **55**, 1666 (1922); R. R. Burtner and J. W. Cusic, *ibid.*, **65**, 262 (1943); H. Raffelson, Dissertation, University of Michigan, 1951, p. 69.

(6) This acid can be prepared readily by the use of the Ivanov reaction. See A. W. Weston and R. W. DeNet, THIS JOURNAL, **73**, 4221 (1951); F. F. Blicke and H. Raffelson, *ibid.*, **74**, 1730 (1952).