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Chloramphenicol[1] (Chloromycetin). IX. Some Analogs Having Variations of the Acyl Group

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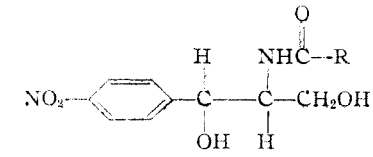
Chloramphenicol has been shown to be the dichloroacetamide of D(levo)-*threo*-1-*p*-nitrophenyl 2-amino-1,3-propanediol.[2,3,4] Certain observations have been made which suggest that the amide linkage in chloramphenicol may play a significant role in the biological function of the compound.[5,6] For this reason it was of interest to prepare a number of analogs having acyl groups other than the dichloroacetyl group. The present paper deals with the preparation of certain amides of DL- and D-(levo)-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol in which the acyl groups are derived from carboxylic acids. These include amides of normal and branched chain aliphatic acids (Table I), amides of halogen substituted aliphatic acids (Table IIa, b and c), and amides of certain aromatic acids (Table III). The availability of these products will now permit studies to be made in which the effect of varying the strength of the carbonyl-to-nitrogen linkage in the amide group can be determined, as well as the influence of variations in the remainder of the acyl group.

When the acid was an α-monohalogen substituted type or halogen substitution was in some position other than α, treatment of the base with the acid chloride in the presence of aqueous alkali was generally the preferred route of synthesis. In some instances partial esterification took place when this method was used. The products of the reaction were then hydrolyzed under conditions designed to cleave acyloxy groups without affecting the amide linkage. A 50% aqueous acetone solution of 0.05 *N* sodium hydroxide at 0° served this purpose. This method of hydrolysis was first used by Kunz and Hudson[7] for determining the O-acetyl groups of carbohydrates.

The above method of acylation was not practical in the preparation of α-bromo or α-iodo acid amides in view of the tendency of these compounds to form morpholone structures upon treatment with aqueous alkali. Reaction of the free base with the α-bromo or α-iodo acid halides in an inert solvent such as ethyl acetate yielded the desired amides. The best yields were obtained by carrying out the reaction in an ethyl acetate–water system in the presence of sufficient sodium bicarbonate to neutralize the acid formed in the reaction.

TABLE I

THE NORMAL AND BRANCHED CHAIN ALIPHATIC ACID AMIDES OF DL- AND D-(LEVO)-*threo*-1-*p*-NITROPHENYL-2-AMINO-1,3-PROPANEDIOL



R	M. p., °C.	Mol. wt.	Analyses, % Theoretical C	H	N	Found C	H	N	Method of prepn.
CH_3— (DL)[8]	166–167	254.2	51.97	5.52	11.03	52.22	5.87	11.26	II
CH_3— (D)[2]	125–126	254.2	51.97	5.52	11.03	51.98	5.49	11.15	II
CH_3CH_2— (DL)	131–132	268.2	53.73	6.01	10.44	53.98	6.25		II
CH_3CH_2— (D)	108–109	268.2	53.73	6.01	10.44	53.44	5.92	10.57	II
$CH_3CH_2CH_2$— (DL)	130–131	282.3	55.31	6.43	9.92	55.05	6.66	9.74	II
$(CH_3)_2CH$— (DL)	147–148	282.3	55.31	6.43	9.92	55.31	6.63	10.15	II
$(C_2H_5)_2CH$— (DL)	127–128	310.3	58.05	7.14	9.02	58.03	6.98	8.80	IIIc
$(CH_3)_2CHCH_2$— (DL)	125–126	296.3	56.74	6.80	9.45	56.98	6.91	9.26	IIIc
$(CH_3)_3C$— (DL)	112–113	296.3	56.74	6.80	9.45	57.10	6.66	9.33	IIIc
$CH_3CH{=}CH$— (DL)	129–130	280.3	55.71	5.75	10.00	55.76	5.99	10.18	IIIa

Amides were prepared by a variety of methods, the particular approach depending upon the nature of the acid. When the acid was of the α,α-dihalogen or α,α,α-trihalogen type, the amide was best prepared by treatment of the free base with the methyl or ethyl ester of the acid.

The α,β-dichloro and α,β-dibromopropionic acid amides showed a strong tendency to lose hydrogen halide when treated with dilute alkali and it was therefore found preferable to prepare these analogs under neutral conditions also. For evidence that the alkali treated products were α,β-unsaturated acid amides, the author is indebted to Dr. J. M. Vandenbelt of these labora-

(1) Chloramphenicol is the antibiotic drug for which Parke, Davis & Company has adopted the trademark, Chloromycetin.

(2) Rebstock, Crooks, Controulis and Bartz, THIS JOURNAL, **71**, 2458 (1949).

(3) Controulis, Rebstock and Crooks, *ibid.*, **71**, 2463 (1949).

(4) Long and Troutman, *ibid.*, **71**, 2469 (1949).

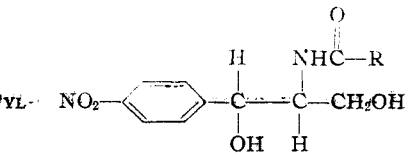
(5) Smith, Worrel and Lilligren, *Science*, **110**, 297 (1949).

(6) Smith and Worrel, *Arch. Biochem.*, **23**, 341 (1949).

(7) Kunz and Hudson, THIS JOURNAL, **48**, 1982 (1926).

TABLE IIa

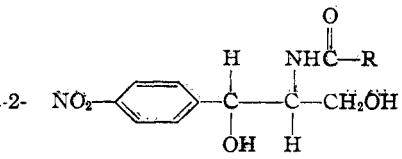
CHLORINE SUBSTITUTED ACID AMIDES OF DL AND D-(LEVO)-*threo*-1-*p*-NITROPHENYL-2-AMINO-1,3-PROPANEDIOL



R	M. p., °C.	Mol. wt.	Analyses, % Theoretical C	H	N	Found C	H	N	Method of prepn.
CH_2Cl— (DL)	99–100	288.7	45.76	4.54	9.70	45.74	4.65	9.45	IIIc
CH_3CHCl— (DL)	138–139	302.7	47.61	4.99	9.25	47.83	5.25	9.54	IIIc
CH_3CH_2CHCl (DL)	92–93	316.7	49.29	5.41	8.84	49.20	5.40	8.96	IIIc
CH_3CCl_2— (DL)[8]	119–120	337.1	42.75	4.19	8.31	42.90	4.30	8.50	I
$(CH_3)_2CCl$— (DL)	124–125	316.7	49.29	5.41	8.84	49.31	5.44	9.16	IIIc
CCl_3— (DL)	148–149	357.6	36.94	3.10	7.85	37.17	3.43	7.92	I
CH_2ClCH_2— (DL)	155–156	302.7	47.61	4.99	9.25	48.04	5.23	9.11	IIIb
$CH_3CHClCH_2$— (DL)	103–104	316.7	49.29	5.41	8.84	49.65	5.58	8.61	IIIc
$CH_2ClCHCl$— (DL)[9]	148–149	337.1	42.75	4.19	8.31	43.06	4.33	8.31	IIIa
$CH_2{=}CCl$— (DL)	122–123	300.7	47.92	4.36	9.32	47.57	4.65	9.32	IIIa, IIIc
CCl_3— (D)	101–102	357.6	36.94	3.10	7.85	37.12	3.25	8.25	I

TABLE IIb

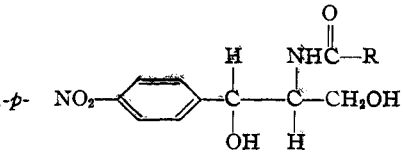
BROMINE SUBSTITUTED ACID AMIDES OF DL AND D-(LEVO)-*threo*-*p*-NITROPHENYL-2-AMINO-1,3-PROPANEDIOL



R	M. p., °C.	Mol. wt.	Analyses, % Theoretical C	H	N	Found C	H	N	Method of prepn.
CH_2Br— (DL)	130–131	333.1	39.90	3.93	8.41	40.08	4.25	8.44	IIIb
CH_3CHBr— (DL)	159–160	347.2	41.51	4.36	8.07	41.75	4.37	8.30	IIIb
CH_3CH_2CHBr— (DL)	123–125	361.2	43.22	4.75	7.75	43.38	5.03	7.95	IIIa
$(CH_3)_2CBr$— (DL)	112–113	361.2	43.22	4.75	7.75	43.39	4.94	7.72	IIIb
$CH_2BrCHBr$— (DL)	155–157	426.1	33.82	3.31	6.57	33.61	3.40	6.77	IIIa
$CH_2{=}CBr$— (DL)	125–126	345.1	41.76	3.80	8.11	41.74	3.70	8.08	IIIa, IIIc
$CHBr_2$— (DL)	152–153	412.1	32.06	2.93	6.80	31.94	3.10		I
$CHBr_2$— (D)	152–153	412.1	32.06	2.93	6.80	32.18	3.05		I

TABLE IIc

FLUORINE AND IODINE SUBSTITUTED ACID AMIDES OF DL AND D-(LEVO)-*threo*-1-*p*-NITROPHENYL-2-AMINO-1,3-PROPANEDIOL



R	M. p., °C.	Mol. wt.	Analyses, % Theoretical C	H	N	Found C	H	N	Method of prepn.
CH_2F— (DL)[10]	120–121	272.2	48.53	4.81	10.28	48.58	4.95	10.55	IIIa
CH_2F— (D)	144–145	272.2	48.53	4.81	10.28	48.64	5.08	10.11	IIIa
CHF_2— (D)[11]	94–95	290.2	45.52	4.17	9.65	45.37	4.26	9.88	I
CF_3— (DL)	103–104	308.2	42.87	3.60	9.09	42.45	3.74	9.41	I
CHFCl— (D)[11]	101–102	306.7	43.08	3.94	9.13	43.23	4.26	9.22	I
CF_2Cl— (DL)	107–108	324.7	40.69	3.42	8.62	40.84	3.61	8.53	I
CH_2I— (DL)	138–139	380.1	34.75	3.45	7.37	35.27	3.63	7.38	IIIa
CF_3— (D)	128–129	308.2	42.87	3.60	9.09	42.62	3.84		I

(8) α,α-Dichloropropionic acid was prepared by the method of Braun, Jostes and Münch, *Ann.*, **453**, 113 (1927).

(9) α,β-Dichloropropionyl chloride was prepared by the method of Marvel, Dee, Cooke and Cowan, THIS JOURNAL, **62**, 3406 (1940).

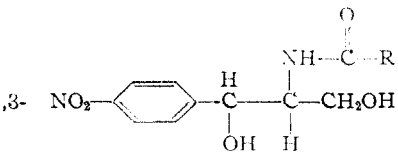
(10) Fluoroacetyl chloride was prepared by the method of Truce, *ibid.*, **70**, 2829 (1948).

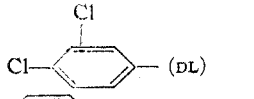
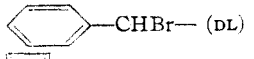
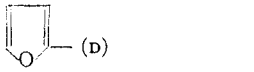
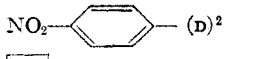
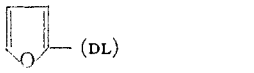
(11) Ethyl fluorochloroacetate and ethyl difluoroacetate were obtained from Dr. Paul Tarrant of the University of Florida.

tories for his studies of the ultraviolet absorption spectra of these compounds and of the crotonic acid amide of the DL-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol base. The absorption spectra of the above compounds were found to be similar, each showing characteristically greater

TABLE III

AROMATIC ACID AMIDES OF DL AND D-(LEVO)-*threo*-1-*p*-NITROPHENYL-2-AMINO-1,3-PROPANEDIOL



R	M. p., °C.	Mol. wt.	Analyses, % Theoretical C	H	N	Found C	H	N	Method of prepn.
 (DL)	149–151	385.2	49.89	3.66	7.27	49.81	3.88	7.11	IIIc
 (DL)	184–185	409	49.89	4.19	6.84	50.21	4.22	7.07	IIIa
 (D)	150–151	306.3	54.90	4.61		54.92	4.80		IIIc
 (D)[2]	204–205	361.3	53.18	4.18	11.63	53.36	4.05	11.78	IIIc
 (DL)	138–139	306.3	54.90	4.61		55.15	4.67	9.44	IIIc

absorption at *ca.* 245 mμ minimum and greater absorption in the region of 215 mμ than is observed when the acid amide is saturated. The minimum itself is also shifted from the normal position of 237 mμ observed with the saturated amides. The pertinent absorption curves have been plotted in Fig. 1 for reference.

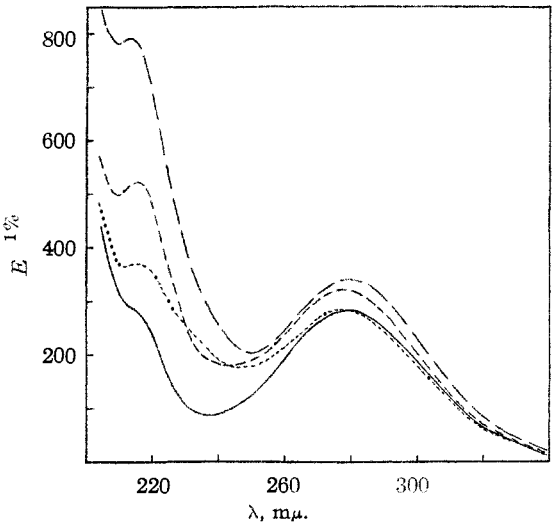


Fig. 1.—Ultraviolet absorption in 0.1 *N* hydrochloric acid of: ——, α,β-dichloropropionic acid amide; — —, crotonic acid amide; — — —, α-chloroacrylic acid amide; and - - -, α-bromoacrylic acid amide of DL-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol.

That the chlorine atom remaining in the compound prepared by alkaline treatment of the α,β-dichloropropionic acid amide was in the α-position was shown directly by preparing the α-chloroacrylic acid amide of the DL-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol base. Since the compounds were identical this preparation constituted a direct proof that dehydrohalogenation does involve the formation of a substituted acrylic acid amide and that the remaining chlorine atom is in the α-position. It may be suggested by analogy that the bromine in the bromoacrylic amide is probably also in the α-position.

The amides of aliphatic acids were best prepared by reaction of the base with the acid anhydrides when these were available. Otherwise, the acid chlorides were used in the presence of aqueous alkali. Due to partial O-acylation which often occurred under these conditions, selective hydrolysis of the reaction products was usually necessary. With the anhydrides the acylation was more easily controlled and recrystallization of the products yielded the desired amides.

Aromatic acid amides were prepared by treatment of the base with the acid chlorides and aqueous alkali.

The foregoing analogs have been tested for antibacterial, antiviral and antifungal properties. The results of these studies will be reported in a subsequent publication.

The author wishes to acknowledge her indebtedness to Drs. Leon A. Sweet and George Rieveschl, Jr., for continued interest and encouragement in this work; to Dr. Harry M. Crooks, Jr., for numerous suggestions and advice; to Dr. J. M. Vandenbelt for the ultraviolet determinations; and to Mr. C. E. Childs and the Misses Geraldine Saladonis and Virginia Pawlik for the many microanalytical determinations.

Experimental

I. **Amide Formation Using Esters (α,α-Dihalogen Substituted Esters and α,α,α-Trihalogen Substituted Esters).**—Either the methyl or ethyl ester of the di- or trisubstituted acid served. The preparation of D-(levo)-*threo*-1-*p*-nitrophenyl-2-difluoroacetamido-1,3-propanediol is taken as an example. A mixture of 1.0 g. of D-base[12] 3 ml. of ethyl difluoroacetate and 10 ml. of absolute

(12) D-Base and DL-base refer to D- and DL-*threo*-*p*-nitrophenyl-2-amino-1,3-propanediol, respectively.

ethanol was refluxed on the steam-bath for 45 minutes. The ethanol was removed at the water-pump and residue treated with 30 ml. of low boiling petroleum ether to remove excess ester. The petroleum ether was decanted and the residue taken into 200 ml. of ethyl acetate. The ethyl acetate was then washed with 0.1 *N* sulfuric acid, 5% sodium bicarbonate and water, and dried over anhydrous magnesium sulfate, and evaporated. The ethyl acetate treatment was not absolutely necessary, but generally gave a product more easily purified by recrystallization. The residue was taken into 15 ml. of ethylene dichloride from which the product crystallized. A yield of 1.1 g. of amide melting at 90–92° was obtained. A sample was recrystallized from ethylene dichloride, ethyl acetate–low boiling petroleum ether, and finally from ethylene dichloride to a melting point of 94–95°.

II. Amide Formation Using Acid Anhydrides.—Anhydrides were preferred as acylation agents to the corresponding acid chlorides. To prepare D-(levo)-*threo*-1-*p*-nitrophenyl-2-propionamido-1,3-propanediol, a sample of 1.0 g. of D-base was heated with 3 ml. of propionic anhydride on the steam-bath for 20 minutes. Ice-water (50 ml.) was added to the cooled solution to decompose the excess propionic anhydride. After one hour the reaction mixture was extracted with three portions of ethyl acetate. The combined extracts were washed successively with 5% sodium bicarbonate, and water, and evaporated to a gum. Since partial esterification sometimes occurs under these conditions, the gum was treated with a mixture of 100 ml. of acetone and 100 ml. of 0.1 *N* sodium hydroxide according to the procedure developed by Kunz and Hudson for the selective removal of acyloxy groups.[7] The reaction mixture was kept at 0° for one hour. Then the solution was neutralized with 5 *N* sulfuric acid and partially evaporated to remove acetone. The aqueous residue was made acidic and extracted with two portions of ethyl acetate. The combined extracts were washed with 0.5% sodium bicarbonate and water and dried, and evaporated. The residue was crystallized from 10 ml. of ethylene dichloride. A yield of 700 mg. of product melting at 105–107° was obtained. Recrystallization from ethylene dichloride, ethyl acetate, and ethylene dichloride raised the melting point to 109–110°.

By heating the base for only ten minutes at 60° with the anhydride, the desired product was obtained without hydrolysis. However, the amides, particularly those of the DL-base, had a tendency to separate before the base had completely dissolved and to crystallize on unreacted particles. These impurities could be removed by dissolving the reaction product in ethyl acetate and washing out the unreacted base with 0.1 *N* sulfuric acid.

IIIa. Amide Formation Using Acid Chlorides.—With the bromo acid halides, monoiodoacetyl chloride and α,β-dihalogen acid chlorides it was preferable to keep the system neutral. Under alkaline conditions when the monobromo or monoiodoacetic acid halides were involved, a secondary reaction occurred in which a morpholone ring was formed by elimination of hydrogen bromide or hydrogen iodide from the molecule. When α,β-dichloro or α,β-dibromopropionic acid chlorides were the acylating agents, the products tended to lose hydrogen halide under alkaline conditions to form α,β-unsaturated acid amides. The preparation of DL-*threo*-1-*p*-nitrophenyl-2-α,β-dibromopropionamido-1,3-propanediol is given as an example to illustrate the conditions which were developed to obtain these amides without secondary reactions. A sample of 1 g. of DL-base was suspended in 15 ml. of ethyl acetate and the mixture chilled to 0°. A volume of 0.5 ml. of α,β-dibromoacetyl chloride was added in portions with vigorous stirring during ten minutes. After standing for thirty minutes longer the reaction mixture was diluted with 50 ml. of ethyl acetate. The ethyl acetate solution was washed successively with 0.1 *N* sulfuric acid and evaporated. The residue was crystallized from 20 ml. of ethylene dichloride. The crude product melted at 133–136° and amounted to 710 mg. The amide was purified to a melting point of 155–157° by twice recrystallizing from ethyl acetate, then from ethylene dichloride.

When this product or the corresponding α,β-dichloropropionic acid amide was treated for one hour at 0° with 0.05 *N* sodium hydroxide in 50% aqueous acetone the elements of hydrogen bromide or hydrogen chloride were removed and the corresponding unsaturated propionic acid amide was obtained.

IIIb.—A somewhat better yield of the amides was obtained by carrying out the reaction in the presence of sodium bicarbonate equivalent to the acid halide. A 5-g. sample of DL-base was suspended in a two-phase system of 25 ml. of distilled water containing 3.96 g. of sodium bicarbonate (two equivalents) and 100 ml. of ethyl acetate. The reaction mixture was cooled to 0° and 4.12 ml. (two equivalents) of bromoacetyl bromide was added in portions with vigorous shaking during fifteen minutes. After standing with intermittent shaking for fifteen minutes longer the ethyl acetate layer was separated and the aqueous phase extracted once again. The combined extracts were washed with 0.1 *N* sulfuric acid, 2% sodium bicarbonate and water, and dried and evaporated. The product was crystallized from 125 ml. of ethylene dichloride to a yield of 3.25 g. of amide which melted at 129–130°.

IIIc.—Excepting the above cases, the most satisfactory method for preparing the amides when the acid chlorides were available was to use the acid chloride in the presence of aqueous alkali. To prepare DL-*threo*-1-*p*-nitrophenyl-2-chloroisobutyramide-1,3-propanediol, 2 g. of DL-base was suspended in a two-phase system consisting of 35 ml. of 0.5 *N* potassium hydroxide and 75 ml. of ethyl acetate chilled to 0°. The reaction was carried out in a separatory funnel which was shaken vigorously throughout. To this system was added 1.5 ml. of α-chloroisobutyl chloride portionwise during ten minutes. The mixture was kept alkaline by adding concentrated alkali as needed. Fifteen minutes after the final addition the solvents were separated. The aqueous layer was extracted once with ethyl acetate. The ethyl acetate extracts were washed with 0.1 *N* sulfuric acid, 5% sodium bicarbonate and water, and dried over anhydrous magnesium sulfate and evaporated. Since some O-acylation usually occurred during this treatment the residue was hydrolyzed by treatment with cold 0.05 *N* sodium hydroxide in aqueous acetone.[7] A yield of 2.3 g. of product was obtained when the amide was isolated from the hydrolysis mixture and crystallized. The product was purified by two recrystallizations from ethylene dichloride to a melting point of 123–124°.

Summary

A number of analogs of chloramphenicol have been prepared in which substitution of a variety of acyl groups for the dichloroacetyl group is made. Amides of normal and branched chain aliphatic acids, halogen substituted aliphatic acids and aromatic acids are described.

DETROIT, MICHIGAN RECEIVED APRIL 3, 1950