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Alkamine Esters of Phenyl-2-thienylacetic Acid and Phenyl-2-thienylglycolic Acid[1]

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A series of alkamine esters of substituted 2-thienylacetic acid and 2-thienylglycolic acid have been prepared by Blicke and Tsao[3] and the antispasmodic activity determined by Lands and co-workers.[3,4,5] This former study of basic alkyl esters included 2-thienyl, phenyl, 2-naphthyl, benzyl and 4-xenyl substituted 2-thienylacetic and 2-thienylglycolic acids. Of this group, the basic esters of the phenyl substituted acids showed the greatest diversity of activity and an enlarged series of esters of these acids have been prepared for pharmacological evaluation.

Both phenyl-2-thienylglycolic acid and phenyl-2-thienylacetic acid were prepared by the method previously described.[3]

The necessary basic alkyl chloride hydrochlorides were prepared by the action of thionyl chloride upon the corresponding basic alkanols in benzene solution. The bromide hydrobromides were prepared by the action of 48% hydrobromic acid on the alcohols in aqueous solution. In order to retard the cyclization of the free halides, ether extracts were used directly with subsequent solvent exchange as required. Most of the basic alkyl ester salts were obtained by refluxing the basic alkyl halide and acid in isopropyl alcohol for fifteen hours[6]; however, some of the esters required special methods as indicated.

We are indebted to Dr. A. M. Lands and coworkers in the Pharmacological Research Laboratories for the preliminary antispasmodic screening data reported herein. The compounds were tested by the Magnus technique against acetylcholine and barium chloride induced spasms in isolated strips of rabbit jejunum. In general, the glycolate esters were more anticholinergic than the corresponding acetates, whereas none of the compounds showed any appreciable activity against barium chloride. The modifications of the ester group produced approximately the same degree of difference in activity in each series. Some conclusions on the relationship between structure and activity, which refer equally well throughout each series, can best be summarized in the higher range of the glycolate series.

1. Quaternary salts increase the activity. The methobromides 21 and 27 are more active than the corresponding hydrochlorides 20 and 26.

2. Increasing the length of the carbon chain between the nitrogen and the carbonyl decreases the activity. Compound 20 is more active than compound 23. This relationship, however, is offset by branching. Actually compound 28 is more active than compound 26.

3. Alkyl substitutions on the nitrogen larger than ethyl diminish the activity as shown by comparing compound 20 with 25. Groups larger than isopropyl cause almost complete loss of activity. The four compounds 20, 25, 29 and 34 show this relationship.

4. The over-all effect of the ester group can best be shown by indicating the diminishing activity of compounds 28, 20, 23, 26, 25, 22, 30, 31 and 29. It is interesting that compound 31 with one methyl group on the nitrogen still retained appreciable activity.

Experimental

Phenyl-2-thienylacetyl Chloride.—Phenyl-2-thienylacetic acid was dissolved in ten times the theoretical quantity of purified thionyl chloride. The clear solution was refluxed for fifteen minutes during which time a deep purple color developed. Excess thionyl chloride was removed first by distillation under reduced pressure and finally by the successive addition and distillation of three 5–10-cc. portions of anhydrous benzene. The resulting deeply colored residual oil was used directly without further purification. Several attempts to purify the product by distillation resulted in total decomposition.

Methyl Phenyl-2-thienylacetate.—A solution of 28.2 g. (0.12 mole) of phenyl-2-thienylacetic acid, 400 cc. of anhydrous methanol and 2.0 cc. of 98% sulfuric acid was refluxed for five hours. The solvent was removed by distillation and the residual oil treated with water. The ester was extracted with ether and the extract washed with dilute sodium bicarbonate solution. The extract was dried with anhydrous magnesium sulfate, the solvent removed and the ester distilled; yield 24.6 g. (82.5%), b. p. 157–161° (0.8 mm.), m. p. 71–73° after recrystallization from *n*-heptane.

Anal. Calcd. for $C_{13}H_{12}O_2S$: S, 13.80. Found: S, 13.96.

N-Methyl-N-(2-hydroxyethyl)-phenyl-2-thienylacetamide.—A mixture of 23.2 g. (0.1 mole) of methyl phenyl-2-thienylacetate, 15.0 g. (0.2 mole) of 2-methylaminoethanol and 0.2 g. of sodium methoxide was heated in an oil-bath for two hours at 140–150°. The reaction mixture was dissolved in hot ethanol, cooled, the crystals collected and washed with cold alcohol; yield 20.4 g. (74.2%), m. p. 153–154° after recrystallization from the same solvent.

Anal. Calcd. for $C_{15}H_{17}NO_2S$: N, 5.09. Found: N, 4.83.

2-Methylaminoethyl Phenyl-2-thienylacetate Hydrochloride.—Hydrogen chloride was bubbled into a suspension of 15.2 g. (0.055 mole) of N-methyl-N-(2-hydroxyethyl)-phenyl-2-thienylacetamide in 400 cc. of isopropyl alcohol until all solid had dissolved. The solvent was removed by distillation and the residual gum solidified by rubbing several times with fresh portions of anhydrous ether; yield 10.55 g. (61.5%). (Analysis and properties in Table I.)

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(3) Blicke and Tsao, THIS JOURNAL, **66**, 1645 (1944).

(4) Lands and Nash, *Proc. Soc. Exptl. Biol. Med.*, **57**, 55 (1944).

(5) Lands, Nash and Hooper, *J. Pharmacol. Exp. Therap.*, **86**, vol. 2, 129 (1946).

(6) Horenstein and Pählicke, *Ber.*, **71**, 1654 (1938).

TABLE I

ALKAMINE ESTERS OF PHENYL-2-THIENYLACETIC ACID $C_6H_5CH(2\text{-}C_4H_3S)COOR$

Compd.	R	M. p. or b. p., °C.	Mm.	Formula	Analyses, %[b] Nitrogen Calcd.	Nitrogen Found	Halogen Calcd.	Halogen Found	Antispasmodic activity (Average values)[c] Acetylcholine	Barium chloride
1	$(C_2H_5)_2NCH_2CH_2-$[a]			$C_{18}H_{23}NO_2S\cdot HCl$					1-4M[d]	1-200T to 400T[e]
2	$(C_2H_5)_2NCH_2CH_2-$	197–199	0.2	$C_{18}H_{23}NO_2S$						
3	$(C_2H_5)_2NCH_2CH_2-$	115–117		$C_{18}H_{23}NO_2S\cdot HBr$	3.52	3.52	20.06	20.02	1-2M to 4M	1-40T to 100T
4	$(C_2H_5)_2NCH_2CH_2-$	65–66		$C_{18}H_{23}NO_2S\cdot CH_3Br$	3.40	3.60	19.33	19.26	1-2M to 4 M	
5	$C_5H_{10}N$[f]CH_2CH_2-[a]			$C_{19}H_{25}NO_2S\cdot CH_3Br$					1-2M to 4M	1-200T
6	$(C_2H_5)_2NCH_2CH_2CH_2-$[a]			$C_{19}H_{25}NO_2S\cdot HCl$					1-2M to 4M	1-200T
7	$(C_4H_9)_2NCH_2CH_2CH_2-$[a]			$C_{23}H_{33}NO_2S\cdot CH_3Br$					1-200T to 500T	1-200T
8	$(i\text{-}C_3H_7)_2NCH_2CH_2-$	174–176	0.05	$C_{20}H_{27}NO_2S$						
9	$(i\text{-}C_3H_7)_2NCH_2CH_2-$	99–100		$C_{20}H_{27}NO_2S\cdot HCl$	3.67	3.78	9.28	9.40	1-2M	1-200T to 500T
10	$(CH_3)_2NCH_2CH_2-$	174–176	0.05	$C_{16}H_{19}NO_2S$						
11	$(CH_3)_2NCH_2CH_2-$	113–115		$C_{16}H_{19}NO_2S\cdot HCl$	4.29	4.16	10.88	10.79	1-500T to 1M	1-150T
12	$(CH_3)_2NCH_2C(CH_3)_2CH_2-$	126–128		$C_{19}H_{25}NO_2S\cdot HCl$	3.79	3.75	9.61	9.92	1-3M to 5M	1-100T
13	$(C_4H_9)_2NCH_2CH_2-$	180–183	0.01	$C_{22}H_{31}NO_2S$	3.75	3.65			1-50T to 100T	
14	$(C_2H_5)_2NCH_2CH(CH_3)-$[g]	162–165	0.01	$C_{19}H_{25}NO_2S$	4.23	4.26			1-1M to 2M	1-100T
15	$(CH_3)HNCH_2CH_2-$	132–134		$C_{15}H_{17}NO_2S\cdot HCl$	4.49	4.47	11.37	11.47	1-200T to 400T	1-50T to 100T
16	$(C_6H_5)HNCH_2CH_2-$	164–165		$C_{20}H_{19}NO_2S\cdot HCl$	3.75	3.67	9.48	9.36	1-200T to 500T	1-200T
17	$(C_6H_5)(C_2H_5)NCH_2CH_2-$	158–160		$C_{22}H_{23}NO_2S\cdot HBr$	3.14	3.12	17.90	17.72		
18	$(C_6H_{11})_2NCH_2CH_2-$	184–185		$C_{26}H_{35}NO_2S\cdot HCl$	3.03	2.95	7.67	7.46	1-10T	
19	$(C_6H_{11})HNCH_2CH_2-$	151–152		$C_{20}H_{25}NO_2S\cdot HBr$	3.30	3.25	18.83	18.94		
	ALKAMINE ESTERS OF PHENYL-2-THIENYLGLYCOLIC ACID $C_6H_5COH(2\text{-}C_4H_3S)COOR$									
20	$(C_2H_5)_2NCH_2CH_2-$[a]			$C_{18}H_{23}NO_3S\cdot HCl$					1-60M	1-200T to 400T
21	$(C_2H_5)_2NCH_2CH_2-$	141–142		$C_{18}H_{23}NO_3S\cdot CH_3Br$	3.27	3.40	18.66	18.68	1-50M to 100M	
22	$(C_5H_{10})N$[f]CH_2CH_2[a]			$C_{19}H_{23}NO_3S\cdot HCl$					1-20M to 40M	1-400T
23	$(C_2H_5)_2NCH_2CH_2CH_2-$[a]			$C_{19}H_{25}NO_3S\cdot HCl$					1-30 to 50M	1-200T
24	$(C_4H_9)_2NCH_2CH_2CH_2-$[a]			$C_{23}H_{33}NO_3S\cdot CH_3Br$					1-100T to 400T	1-100T
25	$(i\text{-}C_3H_7)_2NCH_2CH_2-$	156–157		$C_{20}H_{27}NO_3S\cdot HCl$	3.52	3.46	8.91	8.86	1-10M to 20M	1-200T
26	$(CH_3)_2NCH_2CH_2-$	159–161		$C_{16}H_{19}NO_3S\cdot HCl$	4.09	4.06	10.37	10.30	1-30M to 50M	1-200T
27	$(CH_3)_2NCH_2CH_2-$	189–191 dec.		$C_{16}H_{19}NO_3S\cdot CH_3Br$	3.50	3.61	19.97	20.22	1-40M to 80M	1-50T to 100T
28	$(CH_3)_2NCH_2C(CH_3)_2CH_2-$	142–144		$C_{19}H_{25}NO_3S\cdot HCl$	3.64	3.73	9.22	9.31	1-75M to 100M	1-100T
29	$(C_4H_9)_2NCH_2CH_2-$	119–120		$C_{22}H_{31}NO_3S\cdot HCl$	3.28	3.17	8.32	8.22	1-400T	1-400T
30	$(C_2H_5)_2NCH_2CH(CH_3)-$[g]	141–142		$C_{19}H_{25}NO_3S\cdot HCl$	3.65	3.62	9.24	9.22	1-10M to 20M	1-800T
31	$(CH_3)NHCH_2CH_2-$	139–140		$C_{15}H_{17}NO_3S\cdot HBr$	3.76	3.71	21.47	21.59	1-2M to 4M	1-100T to 500T
32	$(C_6H_5)HNCH_2CH_2-$	164–165		$C_{20}H_{19}NO_3S\cdot HCl$	3.60	3.42	9.09	8.82	1-400T	1-200T
33	$(C_6H_5)(C_2H_5)NCH_2CH_2-$	172–173		$C_{22}H_{23}NO_3S\cdot HCl$	3.35	3.45	8.46	8.37		
34	$(C_6H_{11})_2NCH_2CH_2-$	175–176		$C_{26}H_{35}NO_3S\cdot HCl$	2.93	2.84	7.42	7.20	1-100T to 200T	
35	$(C_6H_{11})HNCH_2CH_2-$	194–195 dec.		$C_{20}H_{25}NO_3S\cdot HBr$	3.18	3.41	18.15	18.05		

[a] Prepared by Blicke and Tsao.[3] [b] We are indebted to Elizabeth B. Macks for the analytical data on these compounds. [c] These figures are preliminary results only but are sufficiently accurate to permit a relative comparison of the compounds [d] 1:4,000,000 dilution. [e] 1:200,000–400,000 dilution. [f] $C_5H_{10}N$ = 1-Piperidyl. Compounds 3, 17, 18 and 25 were recrystallized from isopropyl alcohol; compounds 16, 27, 32, 33 and 35 from anhydrous ethanol; compound 4 from anhydrous ethanol and anhydrous ethyl acetate; compounds 8, 19, 21, 28, 31 and 34 from isopropyl alcohol and isopropyl ether; compounds 9, 26 and 30 from anhydrous ethanol and ethyl ether; compounds 11, 12 and 15 from anhydrous ethyl acetate; and compound 29 from ethyl acetate and isopropyl alcohol. [g] Alternative structure [$(C_2H_5)_2NCH(CH_3)-CH_2-$] not excluded. Compounds 9, 12, 13, 14, 19, 25, 26, 28, 29, 30, 31, 32, 33, 34 and 35 were prepared by Method I; compounds 8, 10, 11, 16 and 18 by Method II; compound 17 by Method III; compound 3 by Method IV and compounds 4, 21 and 27 by Method V.

Preparation of Basic Esters and Salts

I. **From Basic Alkyl Halides.**[6]—Equimolar quantities of basic alkyl halide and acid were dissolved in an appropriate volume of isopropyl alcohol and the solution refluxed for fifteen hours. The solvent was removed by evaporation and the solid or gummy residue rubbed with anhydrous ether. The resulting solid was recrystallized from a suitable solvent as indicated in the tables.

II. **From Phenyl-2-thienylacetyl Chloride.**—The crude acid chloride from above was dissolved in anhydrous benzene and treated with an equivalent of the basic alcohol in the same solvent. After refluxing for one hour, the benzene was removed from the slurry by distillation and the residue dissolved in dilute hydrochloric acid. Insoluble material was extracted with ether. The clear solution was made alkaline to litmus with 10% sodium carbonate solution and the liberated basic-ester extracted with ether. The ether extract was dried with anhydrous magnesium sulfate. After filtration the ether was removed by distillation and the residual oil purified by distillation under reduced pressure. Hydrochlorides were obtained either by treating the dried ether extract or an ether solution of the distilled base with dry hydrogen chloride gas.

III. By **Ester Exchange.**—Equivalent amounts of methyl phenyl-2-thienylacetate and the basic alcohol were mixed with 0.1 g. of sodium methoxide and heated at 200° for twenty-four hours. The reaction mixture was cooled, dissolved in isopropyl alcohol and the solution treated with an equivalent amount of 48% hydrobromic acid. The product was worked up as in II.

IV. **Hydrobromides.**—A purified base was dissolved in isopropyl alcohol and treated with the theoretical quantity of 48% hydrobromic acid. The solvent was removed by distillation and the product purified as under I.

V. **Methobromides.**[7]—Either a purified base or a crude base obtained by neutralizing an aqueous solution of a hydrochloride with 10% sodium carbonate was dissolved in anhydrous ethanol. The solution was placed in a pressure bottle, cooled and treated with four to six equivalents of methyl bromide. After standing at room temperature for twenty-four hours the reaction mixture was worked up as under I.

Summary

Twenty-four new alkamine esters of phenyl-2-

(7) Blicke and Maxwell, THIS JOURNAL, **64**, 430 (1942).

thienylacetic acid and phenyl-2-thienylglycolic acid were prepared and characterized. From the results of some preliminary antispasmodic screening, brief conclusions are drawn on the relationship between structure and activity.

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Studies on the Mechanism of the Mannich Reaction. I. Ethylmalonic Acid, A Methynyl Compound

BY ELLIOT R. ALEXANDER AND ELIZABETH J. UNDERHILL

When a compound containing an active hydrogen atom is treated with formaldehyde and ammonia or a primary or secondary amine, the active hydrogen atom is replaced by an aminomethyl group.

$$-\overset{|}{\underset{|}{C}}H + CH_2O + HNR_2 \longrightarrow -\overset{|}{\underset{|}{C}}-CH_2-NR_2 + H_2O$$

This reaction is commonly called the Mannich reaction,[1] and it has been used widely in synthesis. Its mechanism, however, has not been elucidated. Many types of active methylene and methynyl compounds undergo the reaction and it has been run in both acidic and basic media. The purpose of this investigation was to study the kinetics of the reaction of ethylmalonic acid with formaldehyde and dimethylamine.

$$C_2H_5CH(COOH)_2 + CH_2O + (CH_3)_2NH \longrightarrow C_2H_5\overset{COOH}{\underset{COOH}{C}}-CH_2-N(CH_3)_2 + H_2O$$

Ethylmalonic acid, it will be observed, is a compound with only one replaceable hydrogen atom.

Experimental

Materials

Ethylmalonic Acid.—The ethylmalonic acid (m. p. 110–110.5°) used in this investigation was prepared by the saponification of commercial ethyl ethylmalonate.[2] Before commencing the preparation, however, the ester was shaken with half its volume of 25% aqueous potassium hydroxide for one half hour in order to remove any ethyl malonate which might have been present.

Formaldehyde.—In order to depolymerize any polyoxymethylenes present,[3a] commercial 37% formalin was diluted twenty-fold to give a solution approximately 2% in formaldehyde, which was allowed to stand for at least two days. The solution was standardized by the method outlined below.

Dimethylamine.—A solution, 2.616 *N* in dimethylamine, was made up by diluting commercial 25% aqueous dimethylamine. It was standardized with normal hydrochloric acid, using methyl orange as an indicator.

Nessler reagent (K_2HgI_4) was prepared according to the procedure given in the "Handbook of Chemistry and Physics."[4] It was found convenient to prepare it in quantities of 16 liters.

Dimethylaminomethylethylmalonic Acid.—The method used for the preparation of this acid was essentially that of Mannich and Ganz.[5] From 6.6 g. (0.05 mole) of ethylmalonic acid, 10 ml. (0.05 mole) of 22.5% dimethylamine solution, and 4.1 ml. (0.05 mole) of 37% formalin, was obtained 7.3 g. (77%) of the amino-acid, m. p. 100.5–101° (dec.).

Dimethylaminomethanol.—For the preparation of dimethylaminomethanol the procedure of Henry[6] was modified as follows. To 114 ml. (1.5 moles) of 37% formalin cooled in an ice–salt-bath, 270 ml. (1.5 moles) of 25% dimethylamine solution was added dropwise with stirring. The stirring was continued for two and one-half hours after addition was complete. Anhydrous potassium carbonate was then added in small portions until an oily layer formed. This was separated and dried over anhydrous potassium carbonate. During the entire preparation the temperature was kept below 5°, and the product was kept in a refrigerator. The yield of crude, undistilled material was 79 g. (70%), $n^{20}D$ 1.4060 (The refractive index changed to 1.4050 over a period of twenty four hours and then remained constant.). This substance was dissolved in water and analyzed as described for formaldehyde and dimethylamine.

Anal. Calcd. for C_3H_9NO: CH_2O, 40.0; $(CH_3)_2NH$, 60.0. Found: CH_2O, 36.8; $(CH_3)_2NH$, 61.2.

The infrared absorption spectrum of the substance showed only a very weak absorption band in the region characteristic of the OH group.

Procedures

Determination of Formaldehyde.—The determination of formaldehyde was carried out by a modification of the mercurimetric method of Bougault and Gros.[7] To 50 ml. of Nessler reagent was added a sample containing 0.0002 to 0.0006 equivalent of formaldehyde. A precipitate formed at once, the resulting mixture was shaken for five minutes, and it was then acidified by the addition of 30–40 ml. of 2 *N* acetic acid. Twenty-five ml. of 0.1 *N* iodine solution was added immediately and the precipitate was dissolved by agitation. The excess iodine was titrated with 0.1 *N* sodium thiosulfate solution. Care was taken to keep the mixture alkaline until the addition of the acetic acid by adding to the Nessler reagent 2–6 ml. of 10% sodium hydroxide solution in cases where the formaldehyde sample was strongly acidic. Blanks were run and corrections made for the effects of Nessler reagent, buffer, amine, ethylmalonic acid and dimethylaminomethylethylmalonic acid, on the thiosulfate titer. The corrections were not more than a few tenths of a ml. and were in such directions that they tended to cancel each other.

Determination of the Order of Reaction.—To study the kinetics of the formation of dimethylaminomethylethyl-

(1) Blicke in Adams, "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, p. 303.

(2) Gattermann, "Laboratory Methods of Organic Chemistry," The Macmillan Co., New York, N. Y., 1937, p. 255.

(3) (a) Walker, "Formaldehyde," Reinhold Publishing Corp., New York, N. Y., 1944, p. 31; (b) p. 263.

(4) "Handbook of Chemistry and Physics," 27th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1943, p. 1277.

(5) Mannich and Ganz, *Ber.*, **55**, 3486 (1922).

(6) Henry, *Bull. Acad. roy. belg.*, [3] **28**, 355 (1894).

(7) Bougault and Gros, *J. pharm. Chim.*, **26**, 5 (1922) [*C. A.*, **16**, 3281 (1922)].