Physiologically Active Compounds. V.[1a,b] Aminothiol Esters of Substituted Acetic, Chloroacetic, Benzilic, and Related Acids[2]

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Twenty-one salts of aminothiol esters of substituted acetic, chloroacetic, benzilic, and related acids have been synthesized. The acetic and chloroacetic esters were prepared from the appropriate acid chlorides and the aminothiol; the hydroxy esters, by the hydrolysis of the α-chloroaminothiol esters. A method of preparing aminothiol esters of hydroxy acids from the sodium adduct of the ketone in liquid ammonia by treatment with bis-(2,N-N-diethylaminoethyl)thiol carbonate proved to be satisfactory for the benzilic acid ester only. In a CNS test, the order of increasing activity among the salts of the esters is acetic < chloroacetic < α-hydroxy. The greatest activity among the salts of the α-hydroxy esters ($RR'C(OH)COS(CH_2)_xNR_2''$) is shown when $x = 2$ and when R and R' are unsubstituted rings. Four of these latter esters are superior in the CNS test to benactyzine, although the atropine-like activity of two of them exceeds that of this standard.

In view of the high CNS activity shown by two esters prepared in this Laboratory, dimethyl- and diethylaminoethylthiol esters of benzilic acid, a series of similar esters was prepared for testing. The molecule was varied in that of the four bonds of the α carbon atom, two held aryl or cycloalkyl groups, one held a hydrogen or a chlorine atom, or a hydroxyl group, and the fourth held a thiol ester moiety containing two or three methylene groups and the diethylamino group.

Synthesis of Compounds in Table II.—The original sequence employed in the synthesis of the aminothiol esters was (R and R' = aryl groups)

$$R(R')C(OH)COOH \xrightarrow{PCl_5} R(R')C(Cl)COCl \xrightarrow{HS(CH_2)_2N(C_2H_5)_2}$$

$$R(R')C(Cl)COS(CH_2)_2N(C_2H_5)_2 \xrightarrow{EtOH}$$

$$R(R')C(OH)COS(CH_2)_2N(C_2H_5)_2 \cdot HCl$$

TABLE I

AMINOTHIOL ESTER HYDROCHLORIDES OF SUBSTITUTED ACETIC AND CHLOROACETIC ACIDS, $RR'CXCOSCH_2CH_2N(C_2H_5)_2 \cdot HCl$

Compound	R	R'	X	Yield, %	M.p., °C.	Mol. formula	Analyses, %: Calcd. C	Calcd. H	Found C	Found H
101	C_6H_5	C_6H_5	H	79	128.5–130[a]					
102	3-$CH_3C_6H_4$	3-$CH_3C_6H_4$	H	85	143–144	$C_{22}H_{30}ClNOS$	67.41	7.71	67.38	7.71
103	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	H	86	144–145	$C_{22}H_{30}ClNOS$	67.41	7.71	66.62	7.87
104	C_6H_5	C_2H_5	H	51	175–176[b]	$C_{18}H_{30}ClNOS$	64.11	8.50	64.28	8.06
								Cl, 9.96		Cl, 9.82
105	C_6H_5[c]	C_6H_{11}	H	34	162.5–163	$C_{26}H_{39}NO_8S$	59.40	7.48	59.36	7.46
106	C_6H_{11}	C_6H_{11}	H	80	163.5–165	$C_{26}H_{38}ClNOS$	63.88	10.49	63.58	10.44
107		$C_{12}H_8$[d]	H	56	200–201	$C_{26}H_{24}ClNOS$	66.37	6.68	66.46	6.86
108	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	Cl	77	160–161.5	$C_{22}H_{28}Cl_2NOS$	61.96	6.85	62.08	6.97
109	C_6H_5	C_6H_{11}	Cl	74	147–148.5	$C_{20}H_{31}Cl_2NOS$	59.39	7.72	59.08	7.55

[a] Lit. m.p.[15] 129.5–130.5°. [b] Lit. m.p.[8] 106–108°. [c] Citrate (1:1 ratio) since the hydrochloride was hygroscopic. [d] RR'CH— = fluorene-.

Synthesis of Compounds in Table I.—The free acids were treated with thionyl chloride for conversion into the acid chlorides, which formed the ester salts when reacting with 2-diethylaminoethanethiol. Compound 108 was prepared from *p*-tolnic acid by conversion into the α-chloroacetyl chloride by the method of King and Holmes[3] and treatment of the latter with the aminothiol. Compound 109 was prepared from phenylcyclohexylacetic acid by treatment with thionyl chloride and sulfuryl chloride according to the method of Schwenk and Papa.[4] The α-chloroacetyl chloride thus obtained was converted into the ester salt in the usual manner.

This sequence left something to be desired. Yields were low largely because (a) the acid chlorides, with the exception of that of benzilic acid, were used in an impure form (decomposition occurred on distilling *in vacuo*) and (b) the final hydrochloride could only be purified satisfactorily by conversion to the free base which was purified by crystallization, chromatography, or vacuum distillation. To add to the difficulties, the first step was entirely unsatisfactory when a cycloalkyl was substituted for one of the aryl groups. Under these conditions the reaction did not yield a satisfactory chloroacid chloride. Tests with bromine and potassium permanganate indicated the product was unsaturated.

An attempt next was made to apply the method of Selman[5] which may be represented by the equations

(1) (a) This study was supported by Grant B652 from the National Institutes of Health, U. S. Public Health Service; (b) Inquiries should be addressed to the senior author.

(2) For paper IV, see C. A. Buehler, H. A. Smith, K. V. Nayak, and T. A. Magee, *J. Org. Chem.*, **26**, 1573 (1961).

(3) F. E. King and D. Holmes, *J. Chem. Soc.*, 164 (1947).

(4) E. Schwenk and D. Papa, *J. Am. Chem. Soc.*, **70**, 3626 (1948).

(5) S. Selman, "Rearrangements of the Benzilic Acid Type: Preparation and Use of Dialkyl Metal Adducts of Aromatic Ketones," Doctoral Dissertation, The University of Tennessee, Knoxville, Tennessee, 1959.

TABLE II

AMINOTHIOL ESTER HYDROCHLORIDES OF HYDROXY ACIDS, RR'C(OH)COS(CH$_2$)$_x$NR$_2$''·HCl

Compound	R	R'	R''	x	Yield, %	M.p., °C.	Mol. formula	Analyses, %—Calculated—C	Calculated—H	Found—C	Found—H
110	C_6H_5	C_6H_5	CH_3	2	7	153–154	$C_{18}H_{22}ClNO_2S$	61.43	6.30	61.22	6.45
111	C_6H_5	C_6H_5	C_2H_5	2	23	138–139[a]	$C_{20}H_{26}ClNO_2S$	63.22	6.90	63.31	6.90
112	C_6H_5	C_6H_5	[b]	2	17	185–187	$C_{21}H_{26}ClNO_2S$	64.35	6.69	64.06	6.47
113	C_6H_5	C_6H_5	CH_3	3	19[c]	180–182	$C_{19}H_{24}ClNO_2S$	62.36	6.61	62.32	6.89
114	C_6H_5	3-$CH_3C_6H_4$	C_2H_5	2	34	140–141	$C_{21}H_{28}ClNO_2S$	64.02	7.16	63.68	7.19
115	C_6H_5	3-$CH_3C_6H_4$	CH_3	3	24	145–147	$C_{20}H_{26}ClNO_2S$	63.22	6.90	63.24	6.84
116	C_6H_5	3,4-$(CH_3)_2C_6H_3$	C_2H_5	2	10	155–157	$C_{22}H_{30}ClNO_2S$	64.76	7.41	64.64	7.56
117	C_6H_5	3,4-$CH_2O_2C_6H_3$	C_2H_5	2	23	154–156	$C_{21}H_{26}ClNO_4S$	59.49	6.18	59.30	6.40
118	C_6H_5	3,4-$CH_2O_2C_6H_3$	CH_3	3	11	179–181	$C_{20}H_{24}ClNO_4S$	58.59	5.90	58.72	5.81
119	4-$CH_3C_6H_4$	4-$CH_3C_6H_4$	C_2H_5	2	65[d]	166–167	$C_{22}H_{30}ClNO_2S$	64.76	7.41	64.91	7.42
120	C_6H_5	C_5H_9	C_2H_5	2	35[d]	150–151.5	$C_{19}H_{30}ClNO_2S$	61.35	8.13	61.14	8.13
121	C_6H_5	C_6H_{11}	C_2H_5	2	86[d]	174–175.5	$C_{21}H_{32}ClNO_2S$	62.23	8.36	62.23	8.26

[a] This compound containing S^{35} is reported, m.p. 143–146°, *C. A.*, **52**, 17155 (1958); *Zhur. Obshchei. Khim.*, **28**, 635 (1958). [b] Piperidino ester. [c] Free base. [d] Based on corresponding α-chloro derivative.

$$RCOR' \xrightarrow[\text{liquid } NH_3]{Na} R(R')C(ONa)Na \xrightarrow[\text{liquid } NH_3]{CO[SCH_2CH_2N(C_2H_5)_2]_2}$$

$$R(R')C(ONa)C(ONa)[SCH_2CH_2N(C_2H_5)_2]_2 \xrightarrow[\text{liquid } NH_3]{NH_4Cl}$$

$$R(R')C(OH)COSCH_2CH_2N(C_2H_5)_2$$

2-N,N-Diethylaminoethyl thiol benzilate was produced in 62% yield by this method. Unfortunately, slight variations in the nature of R and R' led to unsatisfactory results. 2,3-Dimethylbenzophenone, for example, gave a product which had the composition of the corresponding carbinol. 4,4'-Dimethylbenzophenone gave a product which corresponded to I on the basis of its analysis and infrared spectrum. Acetophenone as well did not give the desired ester, $[(4\text{-}CH_3C_6H_4)_2C(OH)]_2CO$, perhaps because of the formation of the sodium salt of the enolic form.

Finally, success was achieved in preparing the chloroacid chlorides of acids containing a cycloalkyl group by starting with the appropriate substituted acetic acid and proceeding as indicated previously for compound 109 in Table I.

The salts in Table II were obtained from the corresponding chloro derivative essentially by the method of Kolloff, *et al.*,[6] followed by hydrolysis. The free base of the α-hydroxythiol esters, after purification, was converted into the hydrochloride.

Pharmacological Results and Discussion.—The mydriatic activity and cerebral stimulation tests, as listed in Table III, were made in the laboratories of Parke, Davis and Company through the courtesy of Dr. Martin L. Black. The mydriatic assay method involves estimation of the effect of the drug on mouse pupillary size and the duration of this effect relative to the effect and duration produced by atropine sulfate, both being given intramuscularly. For the CNS tests the "jiggle cage"

TABLE III

MYDRIATIC ACTIVITY AND CEREBRAL STIMULATION OF THIOL ACID ESTERS

No.	Mydriatic activity[a]	CNS activity at indicated dosage (mg./kg.)[b]—100	50	25	12.5	6	3	1.5	0.75	0.38	0.20	0.10	0.05
102	...	0	0										
103	...	0	0										
105	...	0	0										
106	...	0	0										
108	<1/250	0	1+										
109	1/60	4+	4+	2+	1+								
110	1/30	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+		
111	1/25	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+		
113	1/60	4+	4+	4+	4+	4+	4+	4+	2+	1+	0		
114	1/60	4+	4+	4+	4+	4+	4+	2+	0				
115	1/100	2+	3+	3+	3+	0							
116	1/60	4+	3+	2+	2+	0							
117	1/60	4+	4+	4+	4+	4+	2+	0					
118	1/60	3+	2+	2+	2+	0							
119	<1/250	1+	1+										
120	1/4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	1+	0
121	1/2	4+	4+	4+	4+	4+	4+	4+	4+	4+	1+		
[c]	1/25	4+	4+	4+	4+	4+	4+	4+	3+	1+			

[a] Effectiveness in extent and duration of dilation of the pupil compared with atropine sulfate as 1 (mice). [b] Three rats (positive controls) were each given a subcutaneous injection of 30 mg./kg. of caffeine and each of 3 negative control animals received the same volume of isotonic saline solution subcutaneously. One of the doses of the test drug was then given to each of 3 test animals; subcutaneously if soluble, orally if not. Recorder responses were counted for 30 min. intervals for the ensuing 2 hr. period and the one that represented peak activity was chosen for analysis. If one lets N_t, N_p, and N_r represent, respectively, the number of responses for treated, positive control, and negative control animals, a score of 1+ to 4+ can be assigned: $(N_t - N_r)/(N_p - N_r) \times 100 = \%\ R$. The score corresponding to $\%\ R$ ranges is 76–100, 4+; 51–75, 3+; 30–50, 2+; and 20–30, 1+. [c] Benactyzine.

(6) H. G. Kolloff, J. H. Hunter, E. H. Woodruff, and R. B. Moffett, *J. Am. Chem. Soc.*, **71**, 3988 (1949).

method on rats, essentially as described by Schulte, *et al.*,[7] was employed. They show that the most active compounds (110, 111, 120, 121) are more effective in the CNS test than benactyzine, although the atropine-like activity of 120 and 121 is considerably higher. It is interesting to note that these compounds have (a) a hydroxyl rather than a chlorine or hydrogen on the α carbon atom (substituted acetic esters show no activity even at the highest concentration), (b) two rather than three methylene groups in the aminoalkyl moiety, and (c) rings containing no substituents attached to the α carbon atom. These results are essentially the same as those observed by Biel, *et al.*,[8] in their study of the oxygen analogs in which the basic moiety was an alkylpiperidyl group. The importance of the unsubstituted rings was also pointed out by Kadin and Cannon,[9] who attribute their effectiveness to the ability "to be in a coplanar or nearly coplanar configuration."

Experimental[10]

Substituted Acetic Acids.—Those not available commercially were prepared as described.

Di-*m*-tolylacetic Acid.—A solution of 5 g. of sodium in 150 ml. of absolute ethanol was added to a solution of 23.8 g. of 3,3′-dimethylbenzil (obtained from the benzoin, which in turn was obtained from the proper aldehyde) in 300 ml. of dry ether. The solution was stirred and allowed to stand at room temperature for 40 hr. Water (500 ml.) was added and the solution was boiled for 30 min. to remove most of the ether and alcohol. After extracting the solution several times with ether, the remaining aqueous layer was acidified, after which the benzilic acid precipitated. Thorough washing and drying gave 20 g. (78%) of product, m.p. 132–133.5° (lit. m.p.,[11] 134–135°).

To 40 ml. of glacial acetic acid were added 2.5 g. of red phosphorus and 0.8 g. of iodine. After the mixture had been standing for 20 min., 1 ml. of water and 17 g. of the benzilic acid were added. After refluxing for 2.5 hr., the excess phosphorus was removed by filtration and the filtrate was poured slowly into a cold, stirred solution of 4 g. of sodium bisulfite in 150 ml. of water. The yellow product, when washed with water and dried, weighed 15.2 g. (95%) and melted at 105–106° (lit. m.p.,[12] 100–102°).

The following compounds were prepared according to literature directions: di-*p*-tolylacetic acid,[12] dicyclohexylacetic acid,[13] and 9-fluorenecarboxylic acid.[14]

Substituted Acid Chlorides.—Obtained by treatment of the acid with thionyl chloride: diphenylacetyl chloride, yield 70%, m.p. 50.5–51.5° (lit. m.p.,[15] 56–57°); di-*m*-tolylacetyl chloride, yield 74%, b.p. 126–127° (0.4 mm.); di-*p*-tolylacetyl chloride, yield 63%, b.p. 140–142° (0.5 mm.). *Anal.* Calcd. for $C_{16}H_{15}ClO$: C, 74.27; H, 5.84. Found: C, 74.36; H, 5.72; phenylcyclopentylacetyl chloride, yield 74%, b.p. 102–106° (0.2 mm.); phenylcyclohexylacetyl chloride, yield 61%, b.p. 102–103° (0.3 mm.); dicyclohexylacetyl chloride, yield 84%, b.p. 98–99° (0.04 mm.). *Anal.* Calcd. for $C_{14}H_{23}ClO$: C, 69.26; H, 9.55. Found: C, 69.46; H, 9.54; 9-fluorenecarbonyl chloride, was used in an unpurified form.

Ester hydrochlorides of substituted acetic acids in Table I were prepared by the method of Clinton and Salvador.[16]

2-N,N-Diethylaminoethyl Phenylcyclohexylthiolacetate Citrate.—The hydrochloride of this ester was so hygroscopic that purification was difficult. The citrate was prepared in ether solution from the free-base ester,[17] which was recovered as an oil. The white solid (2.5 g., 34%, from 3.3 g. of acid chloride) melted at 162.5–163.0°.

Ester Hydrochlorides of Substituted α-Chloro Acids in Table I. Di-*p*-tolylchloroacetyl Chloride.—This compound was prepared essentially by the procedure of King and Holmes.[3] To 15 g. of *p*-tolnic acid under an atmosphere of nitrogen was added 24.8 g. of phosphorus pentachloride with stirring. A vigorous reaction soon subsided and the light brown liquid remaining was heated on a steam bath for 1 hr. After removing the phosphoryl chloride through the use of an aspirator, the remaining oil was dissolved in ligroin and the solution was heated to boiling with 2 g. of Norit and 3 g. of Celite. Filtration and removal of the ligroin *in vacuo* gave 13.5 g. (79%) of an oil which was used without further purification.

Phenylcyclohexylchloroacetyl chloride was prepared by the method of Schwenk and Papa.[4] To 15 g. of phenylcyclohexylacetic acid was added 45 ml. of cold thionyl chloride and the mixture was refluxed for 1 hr., at which time 95 ml. of sulfuryl chloride was added dropwise over a 2 hr. period. A slight evolution of hydrogen chloride occurred then and during most of the remaining 30 hr. of reflux. Distillation gave 17.1 g. (91%) of a product, b.p. 134° (4 mm.).

Anal. Calcd. for $C_{14}H_{16}Cl_2O$: Cl, 26.15. Found: Cl, 26.15.

2-N,N-Diethylaminoethyl Phenylcyclohexylchlorothiolacetate Hydrochloride.—To the chloroacid chloride (6.5 g.) in 50 ml. of dry benzene was added slowly with constant stirring 3.2 g. of N,N-diethylaminoethanethiol in 75 ml. of dry benzene. After stirring for 12 hr. at room temperature, 100 ml. of ether was added. The solid which formed was dried and crystallized from a mixture of acetone, methanol, and ether to give 6.5 g. (74%) of product, m.p. 147–148.5°.

2-N,N-Diethylaminoethyl-di-*p*-tolylchlorothiolacetate hydrochloride was prepared similarly. The chloroacetyl chloride (8 g.) gave 9 g. (77%) of the desired product, m.p. 160–161.5°.

Syntheses from Dialkyl Metal Adducts of Aromatic Ketones. Bis-(2-N,N-diethylaminoethyl)thiolcarbonate Hydrochloride.—Phosgene gas was bubbled into a solution of 10 g. of 2-N,N-diethylaminoethanethiol in 100 ml. of benzene until precipitation of a white solid was complete. After refluxing for 1 hr., the solid hydrochloride, when washed with dry ether and dried, weighed 10.5 g. (80%), m.p. 224–224.5°.

Anal. Calcd. for $C_{13}H_{30}Cl_2N_2O_2S_2$: C, 42.72; H, 8.27. Found: C, 42.72; H, 8.24.

The hydrochloride and 50 ml. of benzene were made alkaline with aqueous sodium hydroxide and the benzene layer was removed. The aqueous layer was extracted with 50 ml. more of benzene, after which the two benzene portions were combined. The carbonate distilled under reduced pressure at 144.5–145.5° (0.45 mm.); $n^{25}D$ 1.5062.

2-N,N-Diethylaminoethyl Thiolbenzilate.—Approximately 100 ml. of ammonia was condensed in a special 200 ml., three-mouthed, round-bottomed flask equipped with a Dry Ice condenser, an ammonia inlet tube, and a magnetic stirrer. Near the bottom of the flask there was attached a stopcock terminated by a 24/40 male joint. To the liquid ammonia were added 1.14 g. of sodium and 3.37 g. of benzophenone in 25 ml. of dry ether. The solution was stirred for 15 min. and was then permitted to flow through the side arm into a flask containing 200 ml. of liquid ammonia and a solution of 5 g. of bis-(2-N,N-diethylaminoethyl)thiol carbonate in 25 ml. of ether, after which the solution was stirred for 4 hr. Upon evaporating the ammonia, 200 ml. of ether was added and the insoluble material was removed by filtration. The ethereal filtrate on evaporation yielded white crystals which weighed 3.5 g. (62%), m.p. 113–114° after two crystallizations from cyclohexane.

Anal. Calcd. for $C_{25}H_{35}NO_2S$: C, 69.93; H, 7.34. Found: C, 70.00; H, 7.29.

Substituted Benzilic Acids.—These and unavailable starting materials were prepared according to literature directions as indicated: 3-methyl and 3,4-dimethylbenzilic acids,[11] benzpiperoin,[18] benzpiperonil,[19] benzpiperonilic acid.[20]

(7) J. W. Schulte, E. C. Reif, J. A. Bacher, Jr., W. S. Lawrence, and M. L. Tainter, *J. Pharmacol. Exptl. Therap.*, **71**, 62 (1941).

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(10) Boiling points are uncorrected. Melting points were obtained with a Fisher–Johns melting point block or a Mel-temp apparatus, each equipped with a partial immersion thermometer.

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(17) S. S. Lieberman, *Farmakol. i. Toksikol.*, **19**, No. 6, 10 (1956); *C. A.*, **51**, 7587 (1957), reported the preparation of the ester, but no physical constants are given in the abstract.

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(19) N. T. Clarke and E. E. Dreger, "Organic Syntheses," Collective Volume 1, John Wiley and Sons, New York, N. Y., 1941, p. 87.

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α-Chloroacid Chlorides of Substituted Benzilic Acids.—3-Methylphenyl phenyl-, 3,4-dimethylphenyl phenyl- and 3,4-methylenedioxyphenyl phenylchloroacetyl chlorides[3]: In these cases, distillation under reduced pressure led to decomposition of the final products. They were therefore freed from ether, subjected to high vacuum for 15 min., and employed without further purification.

Phenylcyclopentylchloroacetyl Chloride.[4]—Phenylcyclopentylacetic acid (10 g.) gave 9.8 g. (78%) of chloroacid chloride, b.p. 124° (0.5 mm.)

Anal. Calcd. for $C_{13}H_{14}Cl_2O$: Cl, 27.58. Found: Cl, 27.80.

Ester Hydrochlorides of Substituted Hydroxyacids in Table II. 3-N,N-Dimethylaminopropylisothiouronium chloride hydrochloride was obtained in 83% yield from 3-chloro-N,N-dimethylpropylamine hydrochloride by the method of Albertson and Clinton,[21] m.p. 159–161°.

Anal. Calcd. for $C_6H_{17}Cl_2N_3S$: C, 30.77; H, 7.32. Found: C, 30.94, H, 7.16.

2-N,N-Dimethylaminopropanethiol[21] was used in the ethereal solution obtained on extraction.

Compounds 110 through 118 in Table II were prepared by identical procedures which may be described by the synthesis of **3-N,N-dimethylaminopropylthiolbenzilate hydrochloride** according to the method of Kolloff, *et al.*[6] To a solution of 8 g. of diphenylchloroacetyl chloride[3] in 50 ml. of anhydrous ether, was added the ethereal extract from the alkaline hydrolysis of 7 g. of 3-N,N-dimethylaminopropyl isothiouronium chloride hydrochloride; an oil precipitated immediately. After refluxing for 1 hr., the reaction mixture was cooled and the ether was decanted. The residual oil was heated on the steam bath for 15 min. with 100 ml. of water and one drop of concd. hydrochloric acid. Upon being cooled and made basic with sodium carbonate, a white solid separated. Several crystallizations from ethanol led to m.p. 85–87°. When hydrogen chloride was passed into a solution of the solid in anhydrous ether, 1.8 g. (19%) of a white solid formed, which melted at 180–182° after several crystallization from ethanol–ether.

Compounds 119, 120 and 121 were prepared from the corresponding α-chloro derivatives by a procedure which may be illustrated for **2-N,N-diethylaminoethyl phenylcyclohexylthiolglycolate hydrochloride**. The α-chlorester hydrochloride (2.5 g.), dissolved in a minimum amount of water, was refluxed for 2 hr., made distinctly alkaline with sodium carbonate solution, and extracted with ether. The dried ethereal solution was acidified with ethereal hydrogen chloride to give a solid which, when crystallized from a mixture of acetone, methanol, and ether, weighed 2.05 g. (86%) m.p. 174–175.5°.

(21) N. F. Albertson and R. O. Clinton, *J. Am. Chem. Soc.*, **67**, 1222 (1945).

Derivatives of (−)-*trans*-2,3-Epoxysuccinic Acid and Some of their Biological Effects

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Symmetrical esters and amides as well as the nitrile have been prepared from the mold metabolite, (−)-*trans*-2,3-epoxysuccinic acid. Opening of the oxirane ring in epoxysuccinic acid and its methyl ester with ammonia and amines to form *erythro*-β-hydroxy-L-aspartic acid and N-substituted analogs is discussed and some products are described.

The three isomers of epoxysuccinic acid (*trans*-racemate and *cis-meso*) have been related to the corresponding tartaric and chloromalic acids.[1–3] The *cis-meso* form has been prepared by hydrogen peroxide oxidation of benzoquinone[4] and by nitric acid oxidation of the macrolide antibiotic, carbomycin,[5] and both the *cis* isomer and the *trans* racemate can be prepared by tungstate-catalyzed hydrogen peroxide oxidation of, respectively, maleic and fumaric acids.[6] To obtain the pure (−)-*trans* isomer, however, a fermentative preparation was used since fairly high yields had been reported from the fermentation of glucose with *Aspergillus fumigatus*.[7–10] We observed yields from this mold of over 20 g./l. of fermentation broth or a 40% molar conversion calculated from the glucose moiety of the sugar in the crude molasses used. Other molds, particularly *Penicillium viniferum* and *Monilia formosa* also produce this acid.[11–15]

The oxirane ring as substituted in epoxysuccinic acid is less reactive to acidic reagents than many epoxides,[16] permitting selective reactions at the carboxyl groups. Thus, either free epoxysuccinic acid or its slightly soluble barium salt, the form in which the acid was isolated from fermentation broths, could be esterified in alcohols with sulfuric acid catalyst. A number of the esters so prepared are listed in Table I.

When (−)-*trans*-epoxysuccinic acid was heated with hydrochloric acid in methanol, dimethyl *erythro*-chloromalate was formed. Treatment of either this ester or dimethyl epoxysuccinate with cold aqueous ammonia led to high yields of the slightly soluble (−)-*trans*-epoxysuccinamide. Similarly other amides were pre-

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