a sirup had $[\alpha]^{28}D$ +7.21° in water (*c*, 1.00). Votoček and Miksic[29] give $[\alpha]^{20}D$ +9.18° in water for this compound. The amount of material available was too small to allow further purification by means of a solid derivative.

D-Allomethylitol.—This compound was prepared by the sodium amalgam reduction[30] of D-allomethylose[31] and melted at 60°; $[\alpha]^{30}D$ −8.62° in water (*c*, 1.00). The constants recorded[30] for this compound are m. p. 62–63°; $[\alpha]^{16}D$ −11 in water.

Summary

D-Lyxomethylitol, L-lyxomethylitol, L-fucitol, L-gulomethylitol and D-rhamnitol have been prepared through their respective acetates by hydrogenolysis of the corresponding mercaptal acetates with Raney nickel. D-Gulomethylitol, L-rhamnitol, D-idomethylitol and L-glucomethylitol were prepared by the addition of methylmagnesium iodide to the appropriate acyclic compounds. D-Allomethylitol was prepared by sodium amalgam reduction of D-allomethylose.

Each of the ω-desoxy sugar alcohols was subjected to the oxidative action of *Acetobacter suboxydans*. An oxidative specificity of uncertain character was displayed by the organism toward the desoxy compounds. D-Lyxomethylitol, L-gulomethylitol and D-rhamnitol were oxidized rapidly and almost completely. L-Lyxomethylitol, D-allomethylitol and L-fucitol were oxidized slowly and gave small but definite amounts of reducing compounds. L-Glucomethyltiol, D-gulomethylitol, D-idomethylitol and L-rhamnitol were not oxidized by the organism.

Amounts of reducing compounds obtained from L-fucitol and L-lyxomethylitol were markedly increased by adding small quantities of assimilable sorbitol to the media and by re-inoculating with active cells of *A. suboxydans* after an initial growth period.

AMES, IOWA RECEIVED MARCH 4, 1949

(29) Votoček and Miksic, *Bull. soc. chim. France*, [4] **43**, 220 (1928).

(30) Iwadare, *Bull. Chem. Soc. Japan*, **17**, 296 (1942).

(31) Levene and Compton, *J. Biol. Chem.*, **116**, 169 (1936).

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, MONSANTO CHEMICAL CO., ST. LOUIS 4, MISSOURI]

Antihistaminic Agents Containing a Thiophene Nucleus

BY L. P. KYRIDES,[1] F. C. MEYER, F. B. ZIENTY, J. HARVEY AND L. W. BANNISTER[2]

Interest in amine derivatives which contain a thiophene nucleus has resulted in the development of two useful antihistaminic agents, N,N-dimethyl-N′-phenyl-N′-(2-thenyl)-ethylenediamine,[3] and N,N-dimethyl-N′-(2-pyridyl)-N′-(2-thenyl)-ethylenediamine (I).[4] The results reported in this paper are an extension of previous studies[5] in the thiophene series. Several phenyl analogs of the thienyl compounds were made for comparison.

Among the new products prepared (Table II), N,N-dimethyl-N′-(5-chloro-2-thenyl)-N′-phenylethylenediamine proved to be the most potent, approximately 125% as active as Antergan in animal tests. In this case the introduction of chlorine into the thiophene ring had a potentiating effect on antihistaminic activity, similar to the observations of previous workers[6] in the pyridine series; Diatrin has been reported by one group to be two-thirds[5] as active as Antergan, while other workers reported it to be as active[7] as Antergan. When chlorine was introduced into the phenyl ring, the activity dropped markedly. The *o*-chlorophenyl derivatives had negligible activity, the *m*-chlorophenyl derivatives were slightly active and the *p*-chlorophenyl derivatives were the most active, but the best compound in this series, N,N-dimethyl-N′-(*p*-chlorophenyl)-N′-(2-thenyl)-ethylenediamine, was only one-half as active as Antergan.

Introduction of a third methyl group in place of the aryl or pyridyl groups, or replacement of the 2-thenyl group by methoxybenzyl practically eliminated activity. Substitution of chlorine or the 4-morpholinyl group for the dimethylamino group produced inactive compounds.

In the Antistin[8] series, the tetrahydropyrimidine analog, 2-(N-benzylanilinomethyl)-1,4,5,6-tetrahydropyrimidine, and the corresponding 2-thenyl derivative, were found to have insignificant activity.

The products were tested for pharmacological activity in the Lilly Research Laboratories.

Several intermediate trisubstituted-ethylenediamines, Table I, were prepared by treating N,N-dimethyl-2-chloroethylamine hydrochloride with the required substituted aniline or aminoheterocycle using known procedures.[9] The secondary

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(3) This compound is the thiophene analog of Antergan, and is known in this country as "Diatrin." For literature references, see (a) Leonard and Solmssen, THIS JOURNAL, **70**, 2064 (1948). The toxicology and pharmacology of Diatrin were discussed recently by (b) Ercoli, Schachter, Hueper and Lewis, *J. Pharmacol.*, **93**, 210 (1948).

(4) This product is the thiophene analog of Pyribenzamine, and is known in this country under the names, "Histadyl" and "Thenylene." For literature references, see Leonard and Solmssen, ref. 3.

(5) Kyrides, Meyer and Zienty, THIS JOURNAL, **69**, 2239 (1947).

(6) Clapp, Clark, Vaughn, English and Anderson, *ibid.*, **69**, 1549 (1947).

(7) Ercoli, Schachter, Leonard and Solmssen, *Arch. Biochem.*, **13**, 487 (1947).

(8) Antistin is 2-(N-benzylanilinomethyl)-2-imidazoline; *cf.* Meier and Bucher, *Schweiz. med. Wochschr.*, **76**, 294 (1946).

(9) Huttrer, Djerassi, Beears, Mayer and Scholz, THIS JOURNAL, **68**, 2001 (1946).

amines then reacted[9] with benzyl chloride, 3,4-dimethoxybenzyl (veratryl) chloride,[10] cyclohexylmethyl bromide,[11] 2-thenyl chloride (2-thienylmethyl chloride) or 5-chloro-2-thenyl chloride.[6]

2-Chloro-N,N-disubstituted-ethylamines were prepared by reaction of ethanolamine with the required halide or halides (in two steps), to form N,N-disubstituted-2-aminoethanols, and treatment of the latter with thionyl chloride in chloroform. These products and their intermediates are shown in Table III.

N,N-Dimethyl-2-thenylamine was isolated from the by-products obtained in the preparation of (I).

Reaction of N,N-dimethyl-N′-cyclohexylethylenediamine with benzyl chloride or with 2-thenyl chloride proved unsuccessful because of the ready formation of quaternary ammonium salts.

TABLE I

N,N-DIMETHYL-N′-SUBSTITUTED-ETHYLENEDIAMINES, $RNHCH_2CH_2N(CH_3)_2$

R	B. p., °C.	Mm.	Formula	Nitrogen, % Calcd.	Found
o-Chlorophenyl	142–143	14	$C_{10}H_{15}ClN_2$	14.1	14.0
m-Chlorophenyl	130–132	4	$C_{10}H_{15}ClN_2$	14.1	14.0
p-Chlorophenyl	130–132	2	$C_{10}H_{15}ClN_2$	14.1	14.1
p-Methoxyphenyl[a]	130–131	3	$C_{11}H_{18}N_2O$	14.4	14.1
4-Methyl-2-pyrimidyl[b]	86–95	1	$C_9H_{16}N_4$	31.1	30.8

[a] B. p. 180–182° (36 mm.). [b] $n^{25}D$ 1.5263.

TABLE II

N,N-DIMETHYL-N′,N′-DISUBSTITUTED-ETHYLENEDIAMINES, $RR'NCH_2CH_2N(CH_3)_2$

R[a]	R′	Base B. p., °C.	Mm.,	Hydrochloride M. p., °C.	Formula	Chloride, % Calcd.	Found
2-Thenyl	Methyl[b]	83–92	4	230–231[c]	$C_{10}H_{18}N_2S\cdot 2HCl$[d]	26.1	25.7
2-Thenyl	*o*-Chlorophenyl	175–176	3.5	184–185	$C_{15}H_{19}ClN_2S\cdot HCl$	10.7	10.7
2-Thenyl	*m*-Chlorophenyl	190–191	3.5	163–165	$C_{15}H_{19}ClN_2S\cdot HCl$	10.7	10.7
2-Thenyl	*p*-Chlorophenyl	184–186	1.5	186–187	$C_{15}H_{19}ClN_2S\cdot HCl$	10.7	10.7
2-Thenyl	*p*-Methoxyphenyl	175–180	1.5	147–148	$C_{16}H_{22}N_2OS\cdot HCl$	10.8	10.9
2-Thenyl	4-Methyl-2-pyrimidyl[e]	147–148	1.2	140–143	$C_{14}H_{20}N_4S\cdot HCl$	11.3	11.7
5-Cl-2-Th	Phenyl[f]	171	2	163.5–164.5	$C_{15}H_{19}ClN_2S\cdot HCl$	10.7	10.7
5-Cl-2-Th	*o*-Chlorophenyl	184–186	2	143–144	$C_{15}H_{18}Cl_2N_2S\cdot HCl$	9.7	9.6
5-Cl-2-Th	*m*-Chlorophenyl	195–197	2	166–168	$C_{15}H_{18}Cl_2N_2S\cdot HCl$	9.7	9.6
5-Cl-2-Th	*p*-Chlorophenyl	197–200	2	186–188	$C_{15}H_{18}Cl_2N_2S\cdot HCl$	9.7	9.7
5-Cl-2-Th	*p*-Methoxyphenyl	178	1.5	108–110	$C_{16}H_{21}ClN_2OS\cdot HCl$	9.8	9.7
5-Cl-2-Th	2-Pyridyl[g]	171–173	1.8	110–111[h]	$C_{14}H_{18}ClN_3S\cdot HCl$	10.7	10.7
Benzyl	*p*-Methoxyphenyl[i]	167–169	0.6	180–182	$C_{18}H_{24}N_2O\cdot HCl$	11.1	10.9
Veratryl	2-Pyridyl[j,k]	200–205	2	179–180	$C_{18}H_{25}N_3O_2\cdot HCl$	10.1	10.1
Cyclohexylmethyl	2-Pyridyl	160–165	.13	225–226	$C_{16}H_{27}N_3\cdot 3HCl$[l]	28.7	28.2

[a] 5-Cl-2-Th = 5-chloro-2-thenyl. [b] $n^{25}D$ 1.5150. [c] With decomp. [d] Dihydrochloride. [e] $n^{25}D$ 1.5662. [f] $n^{25}D$ 1.5887. [g] $n^{25}D$ 1.5863. [h] Ref. 5 gives m. p. 106–108°. [i] $n^{25}D$ 1.5720. [j] This compound is 2892 R. P., Viaud, *Produits Pharmaceutiques*, **2**, 53 (1947). [k] $n^{25}D$ 1.5777. [l] Trihydrochloride.

TABLE III

N-SUBSTITUTED 2-AMINOETHANOLS AND 2-CHLOROETHYLAMINES, $R_1R_2NCH_2CH_2R_3$

R_1[a]	R_2	R_3	B. p., °C.	Mm.	Formula	Nitrogen, % Calcd.	Found
Benzyl	H	OH[b]	110–111	0.5	$C_9H_{13}NO$	9.3	9.2
Benzyl	Benzyl	OH[c]	160–162	1.0	$C_{16}H_{19}NO$	5.8	..
Benzyl	2-Thenyl	OH	166–167	1.0	$C_{14}H_{17}NOS$	5.7	5.7
Benzyl	2-Thenyl	Cl	167–168[d]	...	$C_{14}H_{16}ClNS\cdot HCl$	Cl^-, 11.7	11.7
2-Thenyl	H	OH[e]	111–112	1.3	$C_7H_{11}NOS$	8.9	..
2-Thenyl	2-Thenyl	OH[f]	151–152	1	$C_{12}H_{15}NOS_2$	5.5	5.6
2-Thenyl	2-Thenyl	Cl	147–148[d]	...	$C_{12}H_{14}ClNS_2\cdot HCl$	Cl^-, 11.5	11.3
5-Cl-2-Th	H	OH	132–134	1.3	$C_7H_{10}ClNOS$	7.3	7.9
5-Cl-2-Th	5-Cl-2-Th	OH	204–206	2	$C_{12}H_{13}Cl_2NOS_2$	4.3	4.5
5-Cl-2-Th	5-Cl-2-Th	Cl	179–180[d]	...	$C_{12}H_{12}Cl_3NS_2\cdot HCl$	Cl^-, 9.4	9.3

[a] 5-Cl-2-Th = 5-chloro-2-thenyl. [b] $n^{25}D$ 1.5395; d^{25}_4 1.392. Ref. 24 gives b. p. 164° (22 mm.). [c] $n^{25}D$ 1.5651. Ref. 24 gives b. p. 220–225° (25 mm.). [d] M. p. of hydrochloride. [e] $n^{25}D$ 1.5535. [f] $n^{25}D$ 1.5855.

Experimental[12]

5-Chloro-2-thenyl Chloride.—Prepared by chloromethylation of 2-chlorothiophene, b. p. 83–85° (8 mm.), $n^{25}D$ 1.5730, d^{25}_4 1.386. Ref. 6 reported 67–68° (1 mm.).

N,N,N′-Trimethyl-N′-(2-thenyl)-ethylenediamine.—A slurry of 19.6 g. of N-methyl-2-thenylamine, b. p. 81–85° (18 mm.),[13] and 11.0 g. of N,N-dimethyl-2-chloroethylamine hydrochloride in 100 cc. of xylene was heated

(10) Decker and Pschorr, *Ber.*, **37**, 3404 (1904).

(11) Hiers and Adams, THIS JOURNAL, **48**, 2388 (1926).

(12) All melting points are corrected.

(13) Blicke and Burckhalter, THIS JOURNAL, **64**, 478 (1942), reported b. p. 75–80° (14 mm.). In a preparation of this base by Mr. D. G. Sheets, N-methyldi-(2-thenyl)-amine, b. p. 116–119° (2 mm.), also was isolated. *Anal.* Calcd. for $C_{11}H_{13}NS_2$: N, 6.3. Found: N, 6.3.

at reflux for twelve hours, cooled, diluted with 20 cc. of water and treated with 8.5 cc. of 50% sodium hydroxide solution. The material was stirred for thirty minutes, the layers were separated, the aqueous layer was extracted with xylene and the combined xylene layer and extract distilled through a fractionating column. A considerable amount of N-methyl-2-thenylamine was recovered, and the product was isolated in 40% yield as a colorless liquid.

3-Dimethylaminopropanol.—This material was prepared using a modification of a procedure described for 3-diethylaminopropanol.[14] A mixture of 45 g. (1 mole) of dimethylamine, 116.1 g. (2 moles) of allyl alcohol (Shell Chemical Co.) and 42 g. of flake sodium hydroxide (96–98%) was placed in an autoclave and heated with agitation at 115–120° for twenty hours. The reaction mixture was cooled to 35–40°, the semi-solid mass was dissolved in 200 cc. of water, the layers were separated and the aqueous layer was extracted with three 50-cc. portions of benzene. The combined alcohol layer and extracts were dried over potassium carbonate and fractionated; b. p. 112–113° (150 mm.) and 162–163°[15] under atmospheric pressure, yield, 59–65%.

N,N-Dimethyl-3-chloropropylamine Hydrochloride (II).—A modification of a procedure described for N,N-dimethyl-2-chloroethylamine hydrochloride[16] was used. A solution of 3-dimethylaminopropanol in chloroform was treated with excess thionyl chloride, added gradually at room temperature with cooling, and the reaction was completed by heating the solution to reflux. Most of the chloroform was removed by distillation, toluene was added to induce crystallization, the hygroscopic hydrochloride was filtered off and dried under reduced pressure; yield, 96%; m. p. 142–145° after recrystallization from chloroform–toluene.

Anal. Calcd. for $C_5H_{12}ClN\cdot HCl$: Cl^-, 22.4. Found: Cl^-, 22.3.

N,N-Dimethyl-N'-(2-pyridyl)-trimethylenediamine (III).—Reaction of II with 2-aminopyridine by a known procedure[9] produced 73% of III, b. p. 109–110° (2 mm.), $n^{25}D$ 1.5390.

Anal. Calcd. for $C_{10}H_{17}N_3$: N, 23.4. Found: N, 23.6.

N,N-Dimethyl-N'-(2-pyridyl)-N'-(2-thenyl)-trimethylenediamine (IV).—Reaction of III with 2-thenyl chloride by a known procedure[17] gave 65% of IV, b. p. 170–172° (2 mm.).[18] The hydrochloride melted at 125.5–126.5°.

Anal. Calcd. for $C_{15}H_{21}N_3S\cdot HCl$: Cl, 11.4. Found: Cl, 11.3.

4-[N-(2-Pyridyl)-N-(2-thenyl)-aminoethyl]-morpholine (V).—4-[N-(2-Pyridyl)-aminoethyl]-morpholine, b. p. 127–131° (0.6 mm.),[19] $n^{25}D$ 1.5455, d^{25}_4 1.007, reacted with 2-thenyl chloride as previously described.[17] The base, V, boiled at 174–175° (0.2 mm.). The hydrochloride melted at 195–195.5°.

Anal. Calcd. for $C_{15}H_{21}N_3OS\cdot HCl$: Cl, 10.4. Found: Cl, 10.4.

2-(2-Thenylamino)-pyridine (VI).—The preparation of this compound from 2-thenyl chloride is more direct than the method using 2-thiophenealdehyde.[3a]

To a slurry of 10.9 g. (0.28 mole) of sodium amide[20] in 250 cc. of toluene there was added 33.0 g. (0.35 mole) of 2-aminopyridine. The mixture was heated slowly to boiling, refluxed for three hours, cooled to 100°, and treated with 18.6 g. (0.14 mole) of 2-thenyl chloride, added dropwise during thirty minutes. The mixture then was heated at 105–110° for three hours, cooled to 30° and quenched with sufficient water to dissolve the sodium chloride. The toluene layer was separated and fractionated. Six grams of 2-aminopyridine was recovered, and 15.7 g. (59%) of VI was obtained as a crystalline solid, b. p. 135–138° (1 mm.), m. p. 81–82°[21] after two recrystallizations from aqueous ethanol.

Anal. Calcd. for $C_{10}H_{10}N_2S$: N, 14.7; S, 16.8. Found: N, 14.6; S, 16.6.

N,N-Dimethyl-2-thenylamine (VII).—(a) In the preparation of I from alkali metal derivatives of N,N-dimethyl-N'-(2-pyridyl)-ethylenediamine (VIII) and 2-thenyl chloride, an oil layer separates out between the product and aqueous layers upon quenching of the reaction mixture with water. The oil layer, probably a quaternary salt resulting from reaction of some 2-thenyl chloride with the dimethylamino group of I or VIII, on heating under reduced pressure yielded 14–15% of a colorless liquid, b. p. 67° (17.5 mm.), $n^{25}D$ 1.5166, d^{25}_4 0.977. This material was identified as VII.

Anal. Calcd. for $C_7H_{11}NS$: neut. equiv., 141.2; *MR*, 43.47; N, 9.9; S, 22.7. Found: neut. equiv., 142.2; *MR*, 43.61; N, 9.9; S, 22.5.

The hydrochloride of VII melted at 182.5–184°.

Anal. Calcd. for $C_7H_{11}NS\cdot HCl$: Cl^-, 20.0. Found: Cl^-, 19.7.

(b) To confirm the identity of VII, it was synthesized as follows: To 60.4 g. of 2-thenyl chloride, stirred and cooled in an ice-bath, there was added slowly 200 cc. of 40% aqueous dimethylamine solution. After the vigorous reaction had subsided (five minutes), the mixture was stirred for an additional thirty minutes and treated with excess 50% sodium hydroxide solution. The layers were separated, the aqueous layer was extracted several times with toluene, and the combined product and extracts were distilled, giving 25.7 g. (40%) of VII, b. p. 85° (31 mm.), $n^{25}D$ 1.5164. The hydrochloride, m. p. 183–184.5°, gave a mixed m. p. of 182.5–184° with the hydrochloride prepared under (1) above.

Ethyl N-Benzyl-N-phenylaminoacetate (IX).—A mixture of 90 g. of ethyl N-phenylaminoacetate,[22] 200 cc. of toluene and 31.8 g. of benzyl chloride was stirred at 70–75° for ten hours, treated with 100 cc. of water and 35 cc. of 50% sodium hydroxide solution, and the toluene layer distilled; b. p. 170–172° (2 mm.), yield, 67%.

2-(N-Benzylanilinomethyl)-1,4,5,6-tetrahydropyrimidine (X).—A mixture of 28 g. (0.103 mole) of IX and 68 g. (0.92 mole) of trimethylenediamine was heated under a column, removing the water and ethanol as formed, and refluxed for an additional four hours. The product was fractionated, yielding 70% of X, b. p. 192–195° (2.5 mm.). The monohydrochloride melted at 210–212°.

Anal. Calcd. for $C_{18}H_{21}N_3\cdot HCl$: Cl^-, 11.2. Found: Cl^-, 11.0.

Ethyl N-Phenyl-N-(2-thenyl)-aminoacetate.—An improved procedure for preparation of this ester[5] was developed. A mixture of 40 g. (0.22 mole) of N-phenylaminoacetate, 14.7 g. (0.11 mole) of 2-thenyl chloride, and 100 cc. of toluene was treated as described for IX above; b. p. 179–180° (1.5 mm.), yield, 70%.

2-[N-(2'-Thenyl)-anilinomethyl]-1,4,5,6-tetrahydropyrimidine Hydrochloride (XI).—A mixture of 25 g. (0.09 mole) of ethyl N-phenyl-N-(2-thenyl)-aminoacetate and 61 g. (0.82 mole) of trimethylenediamine was treated as described for X. The crystalline base (undistilled) was converted to the hydrochloride, which was recrystallized from a mixture of methyl isobutyl ketone and methanol; m. p. 221–223°, yield, 73%.

Anal. Calcd. for $C_{16}H_{19}N_3S\cdot HCl$: Cl^-, 11.0. Found: Cl^-, 10.8.

N-Benzylmorpholine.—This product was obtained instead of the expected 2-benzylaminoethanol when 107.1 g.

(14) Hromatka, *Ber.*, **75**, 131 (1942).

(15) von Braun, *ibid.*, **49**, 969 (1916), reported b. p. 163–164°.

(16) Slotta and Behnisch, *ibid.*, **68**, 754 (1935).

(17) Reference 3a, procedure 5.

(18) Reference 3a gives b. p. 171–174° (4 mm.) for this base, and m. p. 122–124° for the hydrochloride. These workers apparently made this compound from the base of II and 2-thenylaminopyridine.

(19) Ref. 9, p. 2000, reported b. p. 136–138° (0.01 mm.).

(20) Vaughn, Vogt and Nieuwland, THIS JOURNAL, **56**, 2120 (1934).

(21) Ref. 3a gives m. p. 78–80°.

(22) Meyer, *Ber.*, **8**, 1156 (1875).

(1 mole) of benzylamine was treated with 40.5 g. (0.5 mole) of ethylene chlorohydrin at 100° for eight hours. The reaction mixture was cooled, treated with excess 50% sodium hydroxide solution, and the amine layer distilled. N-Benzylmorpholine, b. p. 101–103° (1.5 mm.),[23] $n^{25}D$ 1.5131.

Anal. Calcd. for $C_{11}H_{15}NO$: N, 7.9. Found: N, 7.9.

N-Benzyl-N-(2-thenyl)-2-aminoethanol (XII).—2-Thenyl chloride, (39 g., 0.63 mole) was added to 95 g. (0.63 mole) of 2-benzylaminoethanol,[24] keeping the temperature below 100–105°. The mixture was held at 100–105° for six hours, cooled, treated with water and 58 cc. of 50% sodium hydroxide solution, and the non-aqueous layer separated and dried over anhydrous sodium sulfate. On fractionation, XII was obtained in 40% conversion and 79% yield.

(23) Gabriel and Stelzner, *Ber.*, **29**, 2386 (1896), reported b. p. 260–261°.

(24) Rumpf and Kwass, *Bull. soc. chim.*, **10**, 347 (1943).

N-Benzyl-N-(2-thenyl)-2-chloroethylamine Hydrochloride (XIII).—To 40 g. (0.16 mole) of XII in 100 cc. of chloroform there was added a solution of 26 g. (0.22 mole) of thionyl chloride in 75 cc. chloroform, keeping the temperature of the reaction mixture at 35–40°. After one hour at 35–40°, the mixture was heated at reflux (64°) for one hour, cooled, and the solid product filtered off and washed with chloroform. The crystalline XIII was recrystallized from a mixture of benzene and chloroform with the aid of decolorizing charcoal; yield, 68%.

Summary

A number of new compounds related to known products of high antihistaminic activity have been described. N,N-Dimethyl-N′-(5-chloro-2-thenyl)-N′-phenylethylenediamine hydrochloride was found to be the most active of the new compounds prepared.

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[CONTRIBUTION FROM THE DEPARTEMNT OF CHEMISTRY, COLLEGE OF MEDICINE, NEW YORK UNIVERSITY]

Determination of Organic Compounds as Isotopic Derivatives.[1] II. Amino Acids by Paper Chomatographic and Indicator Techniques[2]

BY ALBERT S. KESTON, SIDNEY UDENFRIEND[3] AND MILTON LEVY

In the isotopic derivative method of analysis,[4] as applied to amino acids, the products of protein hydrolysis are quantitatively converted to *p*-iodobenzenesulfonyl derivatives (pipsyl derivatives) with a reagent labeled with I-131. The radioactive pipsyl derivatives are separated and purified using non-isotopic pipsyl derivatives as carriers in sufficient quantity to allow effective purification by crystallizations, distribution between solvents and selective adsorptions. The analytical result is calculated from the specific activity of a pure sample of the carrier isolated from the mixture and does not depend on complete recovery. The considerable losses accepted in the attainment of purity do not affect the quantitative nature of the result.

Considering the amount of materials recovered in a pure form, paper chromatography[5] seems a more efficient method of separating and purifying substances than is recrystallization. It could not be applied to the rather large amounts of material present after the addition of carriers as used in the original isotopic derivative technique.[4] We tried some experiments on the radioactive pipsyl derivatives on paper chromatograms without addition of carriers. All steps were carried out in a standard quantitative way, avoiding all losses.[6] The inconveniences of this standard type of operation, however, led us to the use of pipsyl derivatives labeled with S-35 as "indicators."[6] The added material is called an indicator since its function is to indicate the fraction of unknown recovered and not to provide sufficient material for the required operations as carriers ordinarily do. After the indicator has been added and mixed with the unknown further manipulations need not be quantitative. The essential feature of their use is that the fractional recovery of the indicator at any stage is also the fractional recovery of the compound of interest. No operation capable of significantly separating the two types of isotopic compounds is used. The amount of indicator added initially must of course be known in counts of S-35 for the sulfur labeled indicators or in absolute quantities if the molar radioactivity of the S-35 is known.

The radiation characteristics of I-131 and S-35 are different and it is easy to determine the proportion of the total registered ionizing events in a mixture due to each by the use of aluminum foil filters. The analysis is calculated from the ratio between S-35 counts (y) and I-131 counts (x) in a pure sample of the compound isolated. If T_s is the number of counts added with the indicator (S-35 counts) and C_r is the molar activity (counts per mole) of the I-131 reagent used, there are in the unknown mixture $u = xT_s/yC_r$ moles of compound estimated. Constancy of the ratio x/y in successive portions of a chromatographic band establishes the purity of the derivative in it and fail-

(1) This work was done under a grant from the American Cancer Society to Dr. R. K. Cannan, made on recommendation of the Committee on Growth of the National Research Council.

(2) A preliminary report of some of this material was presented at the meeting of the American Society of Biological Chemists: *Federation Proceedings*, **8**, 213 (1949).

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(4) A. S. Keston, S. Udenfriend and R. K. Cannan, (a) THIS JOURNAL, **68**, 1390 (1946); (b) **71**, 249 (1949).

(5) E. Comsden, A. H. Gordon and A. J. Martin, *Biochem. J.*, **38**, 224 (1938).

(6) A. S. Keston, S. Udenfriend and M. Levy, THIS JOURNAL, **69**, 3151 (1947).