DETECTION AND PAPER CHROMATOGRAPHY OF N-SUBSTITUTED HYDROXY-, 2-HYDROXYETHYL-, 2-CHLOROETHYL-, AND N,N-BIS-(2-HYDROXYETHYL)-DERIVATIVES

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INTRODUCTION

A number of problems under current investigation in our laboratory relate to the metabolism, degradation and mutagenicity of N-substituted 2-hydroxyethyl-, and 2-chloroethyl pesticides, N-hydroxy carbamates and related nitrogen and carbon substituted hydroxy derivatives. The recognition of N-hydroxylation as an important route in the carcinogenicity and chemotherapy of various acetamido and amino derivatives[1–7] as well as carbamates[8–10] is well documented. In addition, both N-hydroxylation and C-hydroxylation have been shown to be metabolic pathways for pesticidal carbamates[11–14] and 2-chlorotriazines[15–22].

These observations have prompted the need to evaluate various categories of detecting reagents as to their possible utility for subsequent differentiation, and identification of the above moieties in a variety of aliphatic, aromatic and cyclic compounds, and concomitantly ascertain the effect of various substituents on nitrogen and carbon such as hydroxy-, 2-hydroxyethyl- and 2-chloroethyl on their chromatographic behavior in several solvent systems.

BOYLAND AND NERY[8, 23] have indicated the utility of pentacyanoammine ferroates for the colorimetric determination of N-hydroxy urethanes. The detection of a number of amino alcohols utilizing ninhydrin has been reported[24]. In the main, except for a number of investigations specifically related to a biochemical problem[24–28], there appears to be a paucity of information concerning the detection and paper chromatographic behavior of N-substituted alcohols[24, 29].

EXPERIMENTAL

One dimensional descending chromatography was employed utilizing Whatman No. 1 filter paper 25 × 30 cm. Acetone solutions (generally 1–10 μl containing 25–100 μg) of test substances were applied at intervals of 2 cm on a line 6 cm from the bottom of the sheet. The development of the chromatograms was performed in conventional all-glass chambers maintained at 25° ± 0.5° with the solvents allowed to descend to approximately 25 cm from the starting line in the course of 5 to 8 h. Following the development, the papers were either dried at 80° for 1 min or air dried overnight in a hood. The spots were located by either spraying or utilizing the "multiple dripping"

procedure of JEPSON AND SMITH[30]. The developing solvent systems utilized in this work were:

(A) *n*-Butanol–acetic acid–water (5:1:4);
(B) *n*-Butanol–ethanol–water (7:1:2);
(C) Isopropanol–ammonia–water (80:5:15).

Detecting reagents

(*1*) *Sodium nitroprusside–acetaldehyde*[26,31]. 5 % sodium nitroprusside and 10 % acetaldehyde in water mixed with an equal volume of 2.0 % aqueous sodium carbonate prior to use.

(*2*) *FCNP reagent*[32,33]. Mixture of equal volumes of 10 % sodium hydroxide, 10 % sodium nitroprusside, 10 % potassium ferricyanide diluted with 3 volumes of water, mixed in equal volumes with acetone prior to use.

(*3*) *Ninhydrin–acetone*[34,35]. 0.2 % solution in acetone.

(*4*) *Ninhydrin–pyridine*. 0.2 % solution in acetone with 5 % pyridine.

(*5*) *Ferric chloride*. 1 % solution in methanol.

(*6*) *Ehrlich's reagent*[35]. 10 % *p*-dimethylaminobenzaldehyde in acetone–conc. hydrochloric acid (1:4).

(*7*) *Cinnamaldehyde*[36]. 2 ml *trans*-cinnamaldehyde in 95 ml ethanol, 18.5 ml water and 6 ml conc. hydrochloric acid.

(*8*) *4-(p-Nitrobenzyl)-pyridine*[37,38]. 5 % solution in acetone applied to chromatograms. After air-drying, 1 *N* potassium hydroxide in 90 % ethanol is applied.

(*9*) *Percheron reagent*[31,39]. 0.5 % barbituric acid in ethanol containing 2 ml 85 % phosphoric acid.

(*10*) *Pyridine–acetaldehyde*[31,40]. Equal volumes of pyridine and acetaldehyde.

(*11*) *Isatin*[31]. 1 % solution in isopropanol containing 1 % pyridine and 1.5 % zinc acetate.

(*12*) *Viles reagent*[41]. 0.5 % copper acetate in ethanol containing 0.1 % dimethylamine and 2 % triethanolamine.

(*13*) *Gibbs reagent*[20,42]. 0.5 % 2,6-dibromo-N-chloro-*p*-quinone imine in dioxane–acetone (4:1).

(*14*) *N,2,6-Trichloro-p-benzoquinone imine*. 5 % solution in dioxane–acetone (4:1).

(*15*) *Fremy's reagent*[43]. 3 % potassium nitrosodisulfonate in 1 *N* sodium hydroxide.

(*16*) *Potassium permanganate*. 1 % aqueous solution.

Multiple sprays

(*17*) *Ninhydrin–ferric chloride*. Reagent (4) followed after air-drying by reagent (5).

(*18*) *Ninhydrin–Ehrlich's*[44]. Reagent (4) followed after air-drying by reagent (6).

(*19*) *Ninhydrin–FCNP*[35]. Reagent (4) followed after air-drying by reagent (2).

Materials

The alkyl-N-hydroxy carbamates (compounds 2, 3, 4 and 5, Table I) were prepared by the procedure of BOYLAND AND NERY[8,23] via the reaction of hydroxylamine with the respective alkyl carbamate. N-Hydroxy-urea (compound 1) was

synthesized by the procedure of DEGHENGI[45] utilizing hydroxylamine and urethane. "Isohydroxy-urea" (O-carbamoyl-hydroxylamine, compound 52) was prepared by the reaction of potassium cyanate and hydroxylamine according to the procedure of KOFOD[46,47]. N-(2-Hydroxyethyl)-urea was prepared according to the method of CHARLTON AND DAY[48] via the reaction of nitrourea and ethanolamine. N-(2-Hydroxyethyl) carbamate was prepared by the reaction of ethanolamine and ethyl chloroformate according to the procedure of BON-ISHAI[49]. 2-Hydroxyethyl-, and 2-chloroethyl carbamates were prepared via the reaction of aqueous ammonia with ethylene carbonate and 2-chloroethyl chloroformate respectively employing the general procedure of CARTER and co-workers[50]. N-(2-Chloroethyl)-urea was prepared according to the method of HYDE *et al.*[51]. Compounds 10, 15–18, 26, 27, 29, 30, 34, 46, 61 and 62 were obtained from Aldrich Chemical Co., Milwaukee, Wisc., U.S.A.; compounds 11, 12, 31, 32, 50, 54, 59, 65, 66, 73–76, N,2,6-trichloro-*p*-benzoquinone imine, 2,6-dibromo-N-chloro-*p*-quinone imine, and isatin from the J. T. Baker Chemical Co., Phillipsburg, N.J., U.S.A.; compounds 6, 21, 27–39, 43–45, 51, 56, 69–71 and 4-(*p*-nitrobenzyl)-pyridine from K & K Laboratories, Inc., Plainview, N.Y., U.S.A.; compounds 22, 35, 40, 41, 49, 52, 53 and 57 from the Distillation Products, Division of Eastman-Kodak Co., Rochester, N.Y., U.S.A.; compounds 13, 42 from Rohm & Haas Co., Philadelphia, Pa., U.S.A.; compound 7 from Pierce Chemical Co., Rockford, Ill., U.S.A.; compound 24 from Union Carbide Corp., Charleston, W.V[illegible] U.S.A.; compound 25 from Abbott Laboratories, Chicago, Ill., U.S.A.; compo[illegible]07 (3-[2-(3,5-dimethyl-2-oxo-cyclohexyl)-2-hydroxyethyl]-glutarimide) from [illegible] Upjohn Co., [illegible]mazoo, Mich., U.S.A.; compound 9 from Chemical Procurement Laboratories, Inc., College Point, N.Y., U.S.A.; compound 60 from Chemirad Corp., East Brunswick, N.J., U.S.A.; potassium nitrodisulfonate from Alfa Inorganics, Inc., Beverley, Mass., U.S.A.

RESULTS AND DISCUSSION

Table I depicts the R_F value and spot colors of N-hydroxy-, 2-hydroxyethyl-, 2-chloroethyl-, and N,N-bis(2-hydroxyethyl)derivatives. Table II illustrates the R_F values and spot colors of miscellaneous nitrogen and carbon substituted derivatives. A number of general observations as to the chromogenic behavior of the above moieties to the variety of detecting reagents employed can be made.

(1) Nitrogen substituted hydroxy-, 2-hydroxyethyl-, and 2-chloroethyl derivatives are, in the main, more reactive than their respective precursors, *e.g.* succinimide, pyrrolidine, urea and ethyl carbamate with the majority of detecting reagents screened (most markedly toward the nitroprusside detector reagents 1 and 2 in addition to reagents 5 and 14). The above nitrogen substituted moieties possess greater chromogenic activity than the majority of carbon and oxygen substituted moieties toward most of the detecting reagents employed.

(2) Detection of N-hydroxyethyl derivatives. In general these derivatives are best detected by the FCNP (reagent 2) or either of the quinone imine type detectors (reagents 13 and 14), the respective precursors are undetected by both of these classes of reagents, *i.e.*, morpholine, succinimide, pyrrolidine, piperidine, hydrazine, acetamide, ethyl carbamate and ethylene imine. The majority of alipi atic and cyclic N-hydroxyethyl derivatives (compounds 13, 14, 15, 17, 18, 20, 21, 24–[illegible]3) are detected as blue or blue-green spots by a nitroprusside type reagent.

TABLE I

R_F VALUES AND SPOT COLORS OF N-HYDROXY-, N-(2-HYDROXYETHYL)-, N-(2-CHLOROETHYL)- AND N,N-BIS
Colors[a] developed after heating at 80° for 1 min

No.	Compound	Detecting reagents							
		U.V.	1	2	3	4	5	6	7
	N-(Hydroxy)-								
1	Urea	—	O	O→R	—	—	P-V	Y[c]	Y
2	Ethyl carbamate	—	O	O-R	—	—	B-V	Y	—
3	*n*-Propyl carbamate	—	O	O-R	—	—	B-V	Y	—
4	*n*-Butyl carbamate	—	O	O-R	—	—	B-V	Y	—
5	2-Chloroethyl carbamate	—	O	O-R	—	—	B-V	Y	Y (wk)
6	Pyrrolidine	—	B	Y-O	—	—	L	Y	Y-Bn
7	Succinimide	—	B-G	B-G	P	P	R	Y	—
8	Phthalimide	—	Y-O	O	P	Bn	R	—	—
9	Naphthylimide	B	—	O	—	—	O	—	—
	N-(2-Hydroxyethyl)-								
10	Amine	—	R	R-P	Y	Y	O	Y	—
11	Methylamine	—	—	L	—	—	O	—	—
12	Isopropylamine	—	—		—	—	O	—	—
13	*tert.*-Butylamine	—		B-G	—		—	—	—
14	Ethylamine	—		L	G	—	O	O	—
15	Triethyl ammonium iodide	—	—	O-B	P (wk)	Gr (wk)	Y	—	—
16	Formamide	—	—	R	P	P	—	Y (wk)	—
17	Acetamide	—	—	B	C	C	—	—	—
18	Hydrazine	—	Bn	C→B	Y	Y	O	Y-O	Y
19	Urea	—	C	C-O	—	—	—	Y	Y
20	Carbamate	—	O	R	—	—	W	—	—
21	Alanine	—	B	L	—	V	C	Y	Y
22	Lactamide	—	—	Bn (wk)	P	P	—	—	—
23	Ethylenediaminetriacetic acid	—	C→Y	Y	P	P	C-O	—	—
24	Ethylenimine	—	—	B (wk)	C	C	—	G-P	B (wk)
25	Cyclohexylamine	—	B	B (wk)	Bn	Bn	—	—	—
26	Piperidine	—	—	B (wk)	Y-Bn	Y	O (wk)	—	—
27	Piperazine	—	B→G	B (wk)	Bn (wk)	Bn (wk)	O	Y (wk)	Y (wk)
28	Pyrazole	—	—	B (wk)	C	—	O	R	—
29	Pyrrolidine	—	B-Gr	B-Gr	Bn-C	Bn	O (wk)	—	—
30	Morpholine	—	B (wk)	Y (wk)	O (wk)	—	—	—	—

(2-HYDROXYETHYL)-DERIVATIVES

8	9	10	11	12	13	14	15	16	17	18	19	Solvents[b] A	B	C
R	—	—	—	—	—	—	W	W	P-V	Y	O-R	0.42	0.30	0.50
—	—	—	W	—	—	—	W	W	P	Y	O-R	0.81	0.77	0.85
—	—	—	W	—	—	—	W	W	P	Y	O-R	0.83	0.80	0.88
—	—	—	W	—	—	—	W	W	P	Y	O-R	0.86	0.83	0.94
B	—	Bn	Gr	—	Bn	Bn	W	W	P	Y	O-R	0.84	0.81	0.89
—	Y (sl)	—	—	—	—	T	T	W	—	O-R	—	0.48	0.21	0.60
—	—	—	Y	—	P	Bn	W	Bn	O	Y	—	0.55	0.50	0.60
—	Y	Bn	Y	Y	Bn	Bn	O	Bn	O	—	O	0.85	0.84	0.43
—	—	—	—	Y	—	—	Y	—	O	—	—	0.90	0.92	0.93
—	W	Bn	Bn (sl)	—	Bn	P	—	Y-Bn	T (sl)	O-R	L-V	0.30	0.19	0.54
—	—	—	O	—	Bn	Bn	—	—	—	—	G (wk)	0.38	0.30	0.80
—	—	—	O	—	Bn	Bn	—		—	—	G (wk)	0.40	0.36	0.86
R (wk)	—	—	—	—	Bn	Bn	W	Y-Bn	O		Y-G (sl)	0.51	0.43	0.86
W	—	O	W	C	T	T	W	O	T	R-Bn	V	0.42	Stk.	0.62
—	—	—	—	—	—	—	—	Y-Bn	Y	Bn	T	0.24	0.17	0.41
—	—	—	—	—	Bn (wk)	Bn (wk)	W	Y-Bn	—	Y (sl)	O	0.30	0.12	0.54
—	W	—	—	—	Bn (wk)	Bn (wk)	B[c]	Y-Bn	—	—	B	0.32	0.15	0.48
O (wk)	O	Y (wk)	O (wk)	Y	Y-Bn	Bn	W	Y-Bn	T (sl)	Y-O	Y	0.63	0.83	0.90
R	C	—	—	—	Bn	Bn	—	Y-Bn	Y-O	C-O	O	0.68	0.55	0.80
T	—	—	—	—	—	W	—	—	—	—	O-R	0.60	0.55	0.80
—	—	Y	—	C	—	—	—	Y	O	—	G (wk)	0.35	0.08	0.32
Bn (wk)	—	—	—	—	—	Bn (wk)	B[c]	Y-Bn	—	C	—	0.29	0.15	0.41
—	W	Y	Gr	Y	Y	Bn	W	Y-Bn	—	B-G	—	0.24	Stk.	0.06
—	—	Y (wk)	—	—	Bn (wk)	Bn (wk)	—	—	O	B-G	O	0.33	0.93	0.91
Bn (wk)	—	C	—	—	P	Bn	W	Y-Bn	O→W	C-G (wk)	O→G	0.60	0.23	0.90
—	—	B (wk)	C (wk)	—	Bn	Bn	—	—	—	—	—	0.56	0.63	0.89
—	—	Y-Bn	C (wk)	—	O-Bn	Bn	—	Y-Bn	O→Y	C→L	B→G	0.28	Stk.	0.63
—	B[c]	—	Y	—	R	Bn	W	—	C	C-O	O	0.39	0.79	0.86
—	—	B (wk)	C (wk)	—	P	Bn	—	—	—	—	—	0.50	0.33	0.85
—	B (wk)	—	C (wk)	—	Bn	Bn	—	—	O	—	—	0.44	0.30	0.79

(continued on p. 288)

TABLE I (continued)

No.	Compound	Detecting reagents							
		U.V.	1	2	3	4	5	6	7
31	Acetone	—	B-G	V	—	O	Y	—	—
32	Phthalimide	—	C (wk)	B-G (wk)	P	P	—	—	—
33	Naphthylamine	—	—	—	—	—	—	—	—
	N-(2-Chloroethyl)-								
34	Amine	—	W	W	P	P	—	Y (sl)	—
35	Triethyl ammonium chloride	—	—	Y	—	—	—	—	—
36	Urea	—	—	O	—	P	—	Y	Y
37	Piperidine	—	Gr	B (wk)	Gr	Gr	—	—	—
38	Pyrrolidine	—	Gr	B	R	Gr	—	—	—
39	Morpholine	—	Gr	B	C	Gr	Y	—	—
	Bis-(2-hydroxyethyl)-								
40	Glycine	—	—	W (wk)	P	P	—	—	—
41	Urea	—	—	—	P (wk)	—	—	—	—
42	*tert.*-Butylamine	—	—	—	—	—	—	—	—
43	Hydrazine		Bn (wk)	P-Bn (wk)	(wk)	Y (wk)	Y-O (wk)	Y[c]	Y[c]
44	Cyclohexylamine		P (wk)		P (wk)	P	Y-O (wk)	—	—
45	Aniline	B		—	P	P	—	G	—

[a] Colors: B = blue; L = lilac; V = violet; P = purple; O = orange; G = green; T = tan; R = rose; C = crimson; Y = yellow; W = white; Bn = brown; Gr = grey; sl = slow; wk = weak; Stk. = streak.
[b] Solvent A: butanol–acetic acid–water (5:1:4); solvent B: butanol–ethanol–water (7:1:2); solvent C: isopropanol–ammonia–water (80:5:14).
[c] Fluorescence after spraying.

(3) Differentiation of N-hydroxy- and N-hydroxyethyl derivatives. N-Hydroxy derivatives of pyrrolidine and phthalimide can be distinguished from the corresponding N-hydroxyethyl derivative by the use of Ehrlich's or Percheron reagents which do not detect the latter N-hydroxyethyl derivatives. The N-hydroxy derivatives tested (compounds 1–6, 8, 9) yielded orange or crimson spots with a nitroprusside reagent in contrast to the blue or blue-green spots formed for a majority of the N-hydroxyethyl derivatives.

(4) N,N-Bis(2-hydroxyethyl) derivatives can be generally differentiated from the analogous monosubstituted N-2-hydroxyethyl derivatives by the use of the nitroprusside reagents 1 and 2 which react with the monosubstituted class, *e.g.*, compounds 12, 18, 19 and 21 and not with the bis(2-hydroxyethyl) derivatives, *i.e.*, compounds 41 and 42, 45. The mono- and bis(2-hydroxyethyl) derivatives can also be distinguished by use of the Percheron reagent (reagent 9). The bis(2-hydroxyethyl) derivatives, *i.e.*, compounds 40–45 yield yellow spots whereas the mono 2-hydroxyethyl derivatives, *i.e.*, compounds 13, 18, 19 and 21 are undetected.

												Solvents		
8	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*A*	*B*	*C*
—	—	—	R	—	B	B	G	Y	—	Y	G	0.86	0.92	0.94
—	—	Y-Bn	—	—	Bn (wk)	Bn (wk)	—	—	—	—	—	0.75	0.25	0.51
—	—	—	—	—	Bn	Y	—	Y-Bn	—	—	—	0.91	0.91	0.90
R (wk)	—	Bn	Bn (wk)	—	P (sl)	P (sl)	—	Y-Bn	Y (sl)	Y (sl)	Y-G (sl)	0.49	0.16	0.39
R (wk)	—	—	—	—	Bn (sl)	Bn (sl)	—	C	—	—	Y-G (sl)	0.52	0.24	0.48
—	—	—	W	—	—	—	—		—	Y-O	O-C	0.77	0.71	0.80
B	Bn	Y	—	Y	Bn	—	—	—	—	—	—	0.67	0.64	0.78
B	—	Bn	G	—	Bn	Bn	—	—	—	—	—	0.62	0.36	0.71
B	—	Bn	Y	—	Y	Bn	—	—	—	—	—	0.40	0.15	0.62
R	Y	—	—	—	Bn (wk)	Bn (wk)	W	Y-Bn		—	—	0.21	0.10	0.41
R	Y	—	—	—	Bn (wk)	Bn (wk)	—		—	—	—	0.58	0.46	0.65
R (wk)	Y	—	—	—	P→Bn	P→Bn	W	Y-Bn	—	C	O-G (wk)	0.45	0.27	0.85
R	Y	—			Bn	Bn	W	Y-Bn	Bn	O-Bn	Bn	0.56	0.77	0.81
R	Y				Bn	Bn	W	Y-Bn	—	Y-Bn	P	0.53	0.17	0.80
R	Y				Bn	Bn	W	Y-Bn	Y	Y	Y	0.80	0.75	0.80

(5) Blocking of both mono and bis nitrogen substituted 2-hydroxyethyl derivatives by etherification resulted in diminution or elimination of their chromogenic activity with the majority of detection reagents tested, *e.g.*, compounds 52, 53 and 56.

(6) Derivatives of urea. The system of choice for distinguishing and separating an admixture of urea, N-hydroxy-urea, "isohydroxy-urea" and N-2-hydroxyethyl-urea was the application of the multiple spray ninhydrin–pyridine followed by ferric chloride (detector 17), separation being accomplished by the use of solvent system A (*n*-butanol–acetic acid–water). Other reagents that can be employed for the differentiation of the above compounds include the use of the following: FCNP, ferric chloride, Ehrlich's reagent or cinnamaldehyde. The isomeric hydroxy-ureas, in addition, can be distinguished by the yellow fluorescence of "isohydroxy-urea" after spraying with the cinnamaldehyde reagent, in contrast to the non-fluorescence of N-hydroxy-urea when treated similarly. N-hydroxyethyl-urea and N,N-bis(2-hydroxyethyl)-urea are distinguishable from the other derivatives of urea tested by the use of the Percheron reagent which detects N-hydroxyethyl-urea as a crimson

TABLE II

R_F VALUE[a] AND SPOT COLORS OF MISCELLANEOUS NITROGEN AND CARBON SUBSTITUTED DERIVATIVES

Colors[b] developed after heating at 80° for 1 min.

No.	Compound	Detecting reagents							
		U.V.	1	2	3	4	5	6	7
46	Ethyl carbamate	—	—	—	—	—	Y	Y	—
47	2-Hydroxyethyl carbamate	—	B	O	—	—	—	Y-G	Bn
48	2-Chloroethyl carbamate	—	—	—	B	—	—	Y	Y
49	Diethyl bicarbamate	—	—	C	—	—	—	Y-O	—
50	Acetamide	—	O	—	—	—	—	—	—
51	α-Hydroxyacetamide	—	—	O	—	—	—	Y	—
52	N-(2-Methoxyethyl)-acetamide	—	O	—	V	—	W	—	—
53	2-Methoxyethylamine	—	O	L	Y	—	O	O	—
54	Urea	—	—	O-C	—	—	W	Y-O	—
55	O-Carbamoyl-hydroxylamine ("isohydroxyurea")	—	C	L	—	—	Y	O[c]	Y[c]
56	1,3-Bis(2-methoxyethyl)-urea	—	—	—	—	—	—	Y (wk)	Y (wk)
57	Carbamoyl choline	—	—	O	(wk)	Gr	—	Y	—
58	Hydrazine	—	Y		Y-Bn	Bn		O	Y
59	Ethyl hydrazinoacetate	—	Y	G	Y	—		Y	—
60	Ethyleneimine	—	Bn		Y	Y	O-R	Y	O-Bn
61	Ethyl (2-hydroxyethyl) sulfide	—	Bn (wk)		—	—	—	—	—
62	2-Hydroxyethyl-2-imidazolidine thione	—	—	V	P (wk)	Y (wk)	R (wk)	Y	Y (wk)
63	2-Hydroxyethyl acetate	—	—	—	—	—	—	—	—
64	2-Chloroethyl acetate	—	—	—	—	—	—	—	—
65	Phthalimide	—	V	Y	—	—	—	—	—
66	N-Chloro-phthalimide	—	G (wk)	G	—	—	—	Y[c] (wk)	—
67	Cyclohexamide	—	—	—	—	—	—	—	Y (wk)
68	Piperidine	—	—	—	V	—	—	—	—
69	3-Hydroxypiperidine	—	B	B	Y-Bn	Y-Bn	R	—	—
70	4-Hydroxypiperidine	—	B	B	Y	Y	R	—	—
71	3-Hydroxypyridine	B	Y	P-B	Y[c]	Y[c]	O	—	—
72	4-Hydroxypyridine	—	Y (wk)	P-B	Bn (wk)	Bn (wk)	Y	—	—
73	2-(2-Hydroxyethyl)-pyridine	—	—	P (wk)	Gr	—	O (wk)	—	—
74	Morpholine	—	V	—	—	V	—	—	—
75	Succinimide		V	Y (wk)	—	—	—	—	—
76	Pyrrolidine	—	B	—	—	—	—	R	—

For footnotes see Table I.

8	9	10	11	12	13	14	15	16	17	18	19	Solvents[c] A	B	C
Bn	—	—	—	—	Y (sl)	—	—	—	—	—	—	0.85	0.81	0.89
—	—	—	—	—	—	—	W	—	—	Y	O	0.61	0.53	0.70
V	—	—	—	—	T	T	—	Y-Bn	—	—	—	0.85	0.84	0.86
—	—	—	—	—	Y (sl)	—	—	Y-Bn	—	Y	Y-O	0.91	0.89	0.93
—	W	—	—	—	—	—	W	—	—	—	—	0.28	0.15	0.41
—	—	R	—	—	T	T	W	Y	—	Y	O	0.49	0.45	0.65
Y	—	C	R	—	Y	—	—	Y	—	—	—	0.40	0.20	0.52
—	—	C	R	—	Y	—	—	Y	T (sl)	C→B	—	0.64	0.44	0.24
Bn	—	—	—	—	Y (sl)	—	—	—	L (sl)	Y (sl)	O-C	0.47	0.32	0.55
—	—	—	—	—	T (wk)	R (wk)	W	Y-Bn	—	Y	—	0.45	0.35	0.54
—	—	—	—	—	T (sl)	—	—	T (wk)	—	—	—	0.61	0.50	0.69
—	Y[c]	—	W	—	—	—	—	—	—	Y	—	0.59	0.42	0.66
Bn	Y	Bn	Bn	—	—	—	—	—	Y	O-[illegible]	[illegible]	0.10	0.00	0.22
—	—	W	—	—	—	T	W	[illegible]	Y	Y	[illegible]→G (sl)	0.31	0.25	0.40
—	Y	Y-Bn	—	Y	—	—	—	—	R (sl)	Bn-G	V→O (sl)	0.30	0.90	0.87
—	—	Bn (wk)	—	—	Y	Bn	—	Y-Bn	—	—	—	0.64	0.75	0.82
—	—	—	—	—	Y	O	—	—	C (sl)	Y	B	0.66	0.64	0.77
Bn (wk)	—	—	—	—	—	Bn (wk)	—	—	—	—	—	0.36	0.22	0.41
—	—	—	Bn (wk)	—	—	—	W (wk)	T (wk)	—	—	—	0.39	0.25	0.45
B[c]	Y	—	Y	—	—	—	—	—	—	Y	—	0.85	0.84	0.80
—	W	Bn (wk)	—	Bn (wk)	W	W	B[c]	R	—	—	—	0.85	0.84	0.80
—	—	—	—	—	—	T (wk)	—	—	—	Y-O	—	0.23	—	0.37
—	—	—	—	—	—	—	—	—	—	—	—	0.56	0.28	0.92
—	—	Bn	B	Y	Y[c]	Y[c]	—	—	C-Bn	Y-Bn	P	0.47	0.72	0.77
—	—	Bn	Bn	—	Y[c]	Y[c]	—	—	C-Bn	Y	V	0.43	0.63	0.71
—	W	B[c]	Y (wk)	—	B[c]	Bn	—	—	O-Bn	Bn (wk)	V	0.73	0.88	0.69
—	B[c]	—	Y (wk)	—	Y (wk)	Bn	—	—	Bn (wk)	Y-B (wk)	P-B	0.03	0.52	0.66
—	W	—	—	Y (wk)	Bn (wk)	Bn (wk)	—	—	—	—	—	0.56	0.86	0.90
—	—	—	—	—	B	—	—	—	—	—	—	0.41	0.52	0.75
—	—	—	Y	—	—	—	—	—	—	—	C (sl)	0.62	0.54	0.67
Y	—	—	R	—	B	—	—	Y-Bn	—	B-G	Y-G (sl)	0.62	0.38	0.71

spot, the bis(2-hydroxyethyl) derivative yellow, whereas the other derivatives of urea are unreactive.

(7) Derivatives and congeners of ethyl carbamate. N-Hydroxy- and N-2-hydroxyethyl derivatives of ethyl carbamate, as well as 2-hydroxyethyl carbamate are distinguished most readily from ethyl carbamate by the use of FCNP, ferric chloride and Ehrlich's reagents (FCNP and ferric chloride being the detectors of choice). Separation of the above derivatives is best achieved with the solvent system C.

(8) Detection of 2-chloroethyl derivatives. The detection of N-substituted 2-chloroethyl derivatives, *e.g.*, urea, ethyl carbamate, piperidine, morpholine and pyrrolidine is best accomplished via the agency of the 4-(*p*-nitrobenzyl)-pyridine reagent and secondarily, by use of the FCNP reagent. The utility of the former reagent has been previously demonstrated toward a variety of nitrogen mustards[37,38]. It is of interest to note that the FCNP reagent is without effect when the 2-chloroethyl moiety is attached to carbon or oxygen as in compounds 64 and 48 (2-chloroethyl acetate and 2-chloroethyl carbamate respectively).

(9) In comparing the reactivities of the functional moieties, *e.g.*, N-hydroxy-, N-2-hydroxyethyl-, N-2-chloroethyl-, and N,N-bis(2-hydroxyethyl) with a variety of detecting reagents the following orders prevail:

(a) for the nitroprusside reagents (1 and 2)
$$N\text{-}OH > N\text{-}CH_2CH_2OH > N\text{-}CH_2CH_2Cl \gg N,N\text{-bis-}(CH_2CH_2OH)$$
(b) for the ninhydrin reagents (3 and 4)
$$(N\text{-}CH_2CH_2OH > N\text{-}CH_2CH_2Cl > N\text{-}OH > N,N\text{-bis-}(CH_2CH_2OH)$$
(c) for the ferric chloride reagent (5)
$$N\text{-}OH \gg N\text{-}CH_2CH_2OH \gg N\text{-}CH_2CH_2Cl \gg N,N\text{-bis-}(CH_2CH_2OH)$$
(d) for the Ehrlich and cinnamaldehyde reagents (6 and 7)
$$N\text{-}OH > N\text{-}CH_2CH_2OH \gg N\text{-}CH_2CH_2Cl \gg N,N\text{-bis-}(CH_2CH_2OH)$$
(e) for the halogenated quinone imine reagents (13 and 14)
$$N\text{-}CH_2CH_2OH > N\text{-}CH_2CH_2Cl > N\text{-}OH > N,N\text{-bis-}(CH_2CH_2OH)$$

Correlation of R_F with structure

The R_F values of the N-hydroxy-, N-2-hydroxyethyl-, N-2-chloroethyl derivatives as well as the respective parent compounds and related congeners are recorded in Tables I and II. They were obtained with one batch of Whatman No. 1 filter paper (untreated) and represent the mean of several determinations (constant to $\pm$ 0.020). The relationship between R_F and structure of the above moieties with both ethyl carbamate and urea appears to be as follows:

(a) For urea and derivatives

N-2-chloroethyl > N-hydroxyethyl > urea > isohydroxy > N-hydroxy

in solvent systems A and C.

(b) For ethyl carbamate and derivatives

ethyl carbamate > N-hydroxy > N-hydroxy ethyl > 2-hydroxyethyl carbamate

in solvent systems B and C.

The introduction of a polar substituent such as an hydroxyl on a nitrogen (*e.g.* N-hydroxy derivatives of urea, ethyl carbamate, succinimide, pyrrolidine and

phthalimide) results in a derivative possessing a lower R_F value than the parent compound in all the solvent systems employed. When a 2-hydroxyethyl group is introduced on nitrogen however, diminution of the R_F value in comparison with the precursor prevailed only in the cases of ethyl carbamate, phthalimide and piperidine. The reverse effect, *i.e.* increase in R_F value, with introduction of the 2-hydroxyethyl moiety was observed for urea, morpholine, pyrrolidine and acetamide. The introduction of two 2-hydroxyethyl, moieties as in N,N-bis(2-hydroxyethyl)-urea, -hydrazine, -cyclohexylamine, and -aniline (compounds 43, 44 and 45) results in the compound possessing a lower R_F value in comparison to the respective mono N-2-hydroxyethyl derivative in all the solvent systems tested.

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SUMMARY

The chromatographic and chromogenic behavior of 76 nitrogen and carbon substituted hydroxy-, 2-hydroxyethyl-, 2-chloroethyl derivatives and related compounds toward 19 detecting reagents and three solvent systems is described.

REFERENCES

1 J. W. CRAMER, J. A. MILLER AND E. C. MILLER, *J. Biol. Chem.*, 235 (1960) 885.
2 N. NELSON AND W. TROLL, *Federation Proc.*, 20 (1961) 41.
3 E. BOYLAND, D. MANSON AND R. NERY, *Biochem. J.*, 86 (1963) 263.
4 H. UEHLEKE, *Biochem. Pharmacol.*, 12 (1963) 219.
5 J. A. MILLER, J. W. CRAMER AND E. C. MILLER, *Cancer Res.*, 20 (1960) 950.
6 J. BOOTH AND E. BOYLAND, *Biochem. J.*, 91 (1964) 362.
7 C. C. IRVING, *Cancer Res.*, 22 (1962) 867.
8 E. BOYLAND AND R. NERY, *Biochem. J.*, 94 (1965) 198.
9 E. BOYLAND, R. NERY, K. S. PEGGIE AND K. WILLIAMS, *Biochem. J.*, 89 (1963) 113.
10 I. BERENBLUM, D. BEN-ISHAI, N. HARAN-GHERA, A. LAPIDOT, E. SIMON AND N. TRAININ, *Biochem. Pharmacol.*, 2 (1959) 168.
11 H. W. DOROUGH AND J. E. CASIDA, *J. Agr. Food Chem.*, 12 (1964) 294.
12 H. W. DOROUGH, N. C. LEELING AND J. E. CASIDA, *Science*, 140 (1963) 170.
13 J. E. CASIDA, *Ann. Rev. Entomol.*, 8 (1963) 39.
14 J. E. CASIDA, *Radiation and Radioisotopes Applied to Insects of Agricultural Importance*, International Atomic Energy Agency, Vienna, 1963, p. 223.
15 P. CASTELFRANCO, C. L. FOY AND D. E. DEUTSCH, *Weeds*, 9 (1961) 580.
16 H. GYSIN AND E. KNÜSLI, *Advan. Pest Control Res.*, 3 (1960) 289.
17 E. LEIGH, D. G. WAGNER AND D. J. LISK, *J. Dairy Sci.*, 48 (1964) 1267.
18 M. MONTGOMERY AND V. H. FREED, *Weeds*, 9 (1961) 231.
19 R. H. HAMILTON, *Weeds*, 12 (1964) 27.
20 D. D. KAUFMAN, P. C. KEARNEY AND T. J. SHEETS, *Science*, 142 (1963) 405.
21 R. H. HAMILTON AND D. E. MORELAND, *Science*, 135 (1962) 373.
22 M. L. MONTGOMERY AND V. H. FREED, *J. Agr. Food Chem.*, 12 (1964) 11.
23 E. BOYLAND AND R. NERY, *Analyst*, 89 (1964) 520.
24 H. K. ZIMMERMAN AND A. COSMATOS, *J. Chromatog.*, 7 (1962) 77.
25 W. F. FISHBEIN AND P. P. CARBONE, *Science*, 142 (1963) 1069.
26 W. F. COULSON, A. D. SMITH AND J. B. JEPSON, *Anal. Biochem.*, 10 (1965) 101.
27 J. B. JEPSON, P. ZALTZMAN AND J. UDENFRIEND, *Biochim. Biophys. Acta*, 62 (1962) 91.
28 E. G. MCGEER AND W. H. CLARK, *J. Chromatog.*, 14 (1964) 107.
29 L. REIO, *J. Chromatog.*, 13 (1964) 475.
30 J. P. JEPSON AND I. SMITH, *Nature*, 172 (1953) 1100.

31 R. C. R. BARRETO AND S. O. SABINO, *J. Chromatog.*, 13 (1964) 435.
32 L. FISHBEIN, *Rec. Trav. Chim.*, 84 (1965) 465.
33 J. ROCHE, *Biochim. Biophys. Acta*, 14 (1954) 71.
34 G. TOENNIES AND J. J. KOLB, *Anal. Chem.*, 23 (1951) 823.
35 I. SMITH, *Chromatographic Techniques*, Interscience, New York, 1958, p. 66.
36 M. P. THOMAS AND H. J. ACKERMANN, *J. Agr. Food Chem.*, 12 (1964) 433.
37 H. G. PETERING AND G. J. VANGIESSEN, *J. Pharm. Sci.*, 52 (1963) 1160.
38 H. M. RAUEN, *Arzneimittel-Forsch.*, 14 (1964) 855.
39 F. PERCHERON, *Bull. Soc. Chim. Biol.*, 44 (1962) 1162.
40 M. FURTH AND H. HERRMANN, *Biochem. Z.*, 280 (1935) 448.
41 F. J. VILES, *J. Ind. Hyg. Toxicol.*, 22 (1940) 188.
42 L. REIO, *Svensk Kem. Tidskr.*, 76 (1964) 5.
43 H. GEHLEN AND G. DASE, *Z. Anorg. Chem.*, 275 (1954) 327.
44 E. VANWINKLE, *J. Chromatog.*, 11 (1963) 272.
45 R. DEGHENGHI, *Organic Syntheses*, Vol. 40, Wiley, New York, 1960, p. 60.
46 H. KOFOD, *Acta Chem. Scand.*, 7 (1953) 274, 938.
47 H. KOFOD, *Acta Chem. Scand.*, 9 (1955) 1575.
48 R. W. CHARLTON AND A. R. DAY, *J. Org. Chem.*, 1 (1936) 552.
49 N. BEN-ISHAI, *J. Am. Chem. Soc.*, 78 (1956) 1964.
50 H. E. CARTER, R. L. FRANK AND H. W. JOHNSTON, *Organic Syntheses*, Coll. Vol. III, Wiley, New York, 1955, p. 166.
51 K. A. HYDE, E. ACTON, W. A. SKINNER, L. GOODMAN, J. GREENBERG AND B. R. BAKER, *J. Med. Pharm. Chem.*, 5 (1962) 1.